TOXICITY AND ESTROGENIC ACTIVITY OF POLYBROMINATED DIPHENYL ETHERS (PBDEs)

FINAL REPORT

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EXECUTIVE SUMMARY

The finding of polybrominated diphenyl ether (PBDE) flame retardants in environments and in organisms throughout the world is attracting international attention. In the Great Lakes region, PBDEs have been detected in air, sediment, surface water, fish, birds, and in human blood. PBDEs have been widely used as flame retardants in plastics, textiles, and furniture since the 1960s. BDE-47 and BDE-99, found in the commercial penta-BDE product, are the congeners detected most frequently and in the highest concentrations in organisms. While we know PBDEs are bioaccumulating in organisms, we know very little about their effects and their sources. The purpose of this research was to 1) determine estrogenic activity of BDE-47 and BDE-99 using the E-screen assay, 2) determine the acute and chronic toxicity of BDE-47 to the aquatic invertebrate, *Ceriodaphnia dubia*, 3) determine the concentration of PBDEs in sediments of the Sheboygan River basin, and 4) determine the concentration of PBDEs in wastewater treatment plant and industrial effluents in the Sheboygan River basin. The E-screen assay utilizes MCF-7 breast cancer cells, which proliferate in the presence of estrogen or estrogen-like compounds. Results of the E-screen assays indicate BDE-47 and BDE-99 can act as estrogen receptor agonists; however, both of these congeners were much less potent agonists than 17β-estradiol. Percent of maximum response for each congener was less than 10% of that of 17β-estradiol. Concentrations of BDE-47 and BDE-99 that caused proliferation in the E-screen assays were six orders of magnitude greater than concentrations of 17β-estradiol that caused proliferation. BDE-47 was toxic to *C. dubia*, with LC_{50} values of 4.60 and 2.95 μg/L. BDE-47 also affected reproduction in *C. dubia*. The chronic toxicity test yielded a seven-day LOEC value of 2.0 μg/L, an NOEC value of 1.4 μg/L, and an MATC value of 1.66 μg/L. The calculated IC_{25} value was 2.05 μg BDE-47/L. Sediments and effluents collected in the Sheboygan River basin were analyzed to determine the concentrations of nine different PBDE congeners: BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, and BDE-154. Only two PBDE congeners were detected in effluents collected from facilities: BDE-47 and BDE-99. Both of these congeners were detected in effluents collected from two wastewater treatment plants and from one industry. Concentrations ranged from below detection to 4.5 and 3.3 ng/L, for BDE-47 and BDE-99, respectively. These concentrations are much lower than those shown to cause acute or chronic toxicity to *C. dubia*, and much lower than those shown to result in estrogenic activity. Six different PBDE congeners were detected in sediments, with concentrations ranging from 0.45 to 6.0 ng/g. BDE-47 and BDE-99 were the congeners detected most frequently, and concentrations of BDE-99 were always slightly higher than those of BDE-47 in each sediment sample where both were detected. BDE-28, BDE-67, and BDE-138 were never detected. PBDEs that accumulate in sediment may be taken up by benthic organisms and passed on through the food chain, as demonstrated by the relatively high concentrations measured in Lake Michigan Chinook and Coho salmon (Manchester-Neesvig et al. 2001). Given the high volume of PBDEs contained in many products currently in use, and the long life of some of those products, it is possible that concentrations of PBDEs in the environment and in organisms will continue to rise for some time to come, despite bans by some states and a phase-out of production by Great Lakes Chemical of the penta- and octa-BDE commercial mixtures. The potential for continued increases in concentrations of the lower brominated congeners, together with recent evidence for uptake and debromination of BDE-209 (the predominant congener in the deca-BDE commercial mixture, the commercial mixture for which no bans or phase-outs are planned) underline the need for continued study of PBDEs.

INTRODUCTION

Problem Definition/Background

The finding of polybrominated diphenyl ether (PBDE) flame retardants in environments and organisms throughout the world is attracting both international and local attention. In the Great Lakes region alone, PBDEs have been detected in air over Lakes Superior, Michigan and Erie (Strandberg et al. 2001), in surface water and sediment of Lake Ontario (Luckey et al. 2001, Alaee 2001), in carp in the Buffalo River (Lognathan et al. 1995), in carp and large mouth bass in the Detroit and Des Plaines Rivers (Rice et al. 2002), in lake trout in all five Great Lakes (Zhu and Hites 2004, Luross et al. 2002), in coho and chinook salmon in Lake Michigan (Manchester-Neesvig et al. 2001), in walleye from Lake Erie (Zhu and Hites 2004), in smelt in Lakes Ontario and Superior (Dodder et al. 2002), in herring gulls throughout the basin (Norstrom, et al. 2002), and in human blood samples collected by a commercial blood collection facility in Illinois (Sjodin et al. 2001). PBDEs have been widely used as flame retardants in plastics, textiles, and furniture since the 1960s. Three different commercial PBDE products are available: pentaBDE, octaBDE and decaBDE. Each is made up of a mixture of different brominated diphenyl ether (BDE) congeners. BDE-47 and BDE-99, found in the pentaBDE product, are the congeners detected most frequently and in the highest concentrations in organisms. PBDEs are similar in structure to polychlorinated biphenyls (PCBs) and studies have shown that while concentrations of PBDEs are currently much lower than concentrations of most PCBs in organisms, uptake efficiency for some PBDEs is greater than for some PCBs (Gustafsson et al. 1999). While we know PBDEs are bioaccumulating in both terrestrial (including human) and aquatic organisms in the Great Lakes region, we know relatively little about the toxicity, carcinogenicity, and endocrine disrupting potential of these compounds.

Some PBDEs may be developmental neurotoxins. In studies conducted by Eriksson et al. (2001), neonatal exposure of mice to BDE-99 adversely affected memory and learning, and neonatal exposure to BDE-47 and to BDE-99 adversely affected behavior. Branchi et al. (2001) also observed adverse effects of BDE-99 on behavior in mice.

Carcinogenicity has been studied in animals for only the decaBDE congener, BDE-209. This congener was shown to be carcinogenic in mice at high doses (in Hooper and McDonald 2000) and is currently listed as a class C compound (possible human carcinogen) in EPA's IRIS (Integrated Risk Information System) database. However, an epidemiologic study showed an association between adipose tissue levels of a tetraBDE congener, BDE-47, and the risk of non-Hodgkin lymphoma (Hardell et al. 1998).

Some studies have shown some PBDEs induce dioxin-like, or aryl hydrocarbon (Ah) receptor-mediated activity (Bunce et al. 2001, Van Overmeire et al. 2001, Meerts et al. 1998 in Meerts et al. 2001). Meerts et al. (1998) examined Ah receptor-mediated activity of 17 different PBDE congeners using an Ah-CALUX assay. Of the 17 PBDE congeners tested, BDE-153, BDE-166 and BDE-190 exhibited the highest activity, although activity levels observed were several orders of magnitude lower than that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Activation of the Ah receptor can cause induction of the cytochrome P-450 isozyme CYP 1A1, a biotransformation enzyme. In contrast, other studies have shown some PBDEs *inhibit* dioxin-like

activity (Kuiper et al. 2004, Tomy et al. 2004). Kuiper et al. (2004) observed significant reduction of TCDD-induced EROD activity in the presence of BDE-47, BDE-99 and BDE-153 and cautioned that like PCBs, PBDEs can interfere with determination of EROD activity (i.e., obscure the presence of Ah receptor agonists such as TCDD) in environmental samples. Tomy et al. (2004) suggest the induction of Ah receptor-mediated activity observed in some studies may have be due to the presence of impurities (e.g., PBDDs and PBDFs) in the commercial PBDE mixtures studied.

Orn and Klasson-Wehler (1998) showed that BDE-47 can be biotransformed to hydroxylated PBDEs in rats and mice. Hydroxylated PBDEs have also been found in blood plasma of Baltic salmon, although the exact source of these compounds is unknown (Asplund et al. 1999). Several hydroxylated PBDEs have been shown to bind competitively to the thyroid hormone receptor (Marsh et al. 1998 in Meerts et al. 2001), and to transthyretin (Meerts et al. 2000). Commercial pentaBDE has been shown to reduce thyroid hormone levels and increase thyroid hyperplasia in rats, and to reduce T4 levels in mice. BDE-47 has also been shown to reduce thyroid hormone levels in rats, while BDE-209 has been shown to increase thyroid hyperplasia and the incidence of thyroid tumors in mice (in Hooper and McDonald 2000).
While the effects of PBDEs on thyroid hormones have been well studied, little is known about their effects on other aspects of endocrine systems, such as estrogen receptors. Meerts et al. (2001) investigated the estrogenic and antiestrogenic activity of 17 different PBDE congeners *in vitro*, using a luciferase-based ER-CALUX assay. Eleven of the 17 congeners exerted some estrogenic activity, with the highest estrogenic activity observed for BDE-100, BDE-75 and BDE-51. Because BDE-47 and BDE-99 are the two congeners detected most frequently and in the highest concentrations in organisms and in the environment, more information on the estrogenic activity of these two congeners is needed.

Information on the toxicity of PBDEs to aquatic organisms is also scarce. BDE-47 adversely affected development rate of a marine copepod (Breitholtz et al. 2001) and BDE-99 was acutely toxic to the freshwater cladoceran, *Daphnia magna* (Evandri et al. 2003). While data on the toxicity of the higher brominated congeners (e.g., BDE-209) to aquatic organisms are not available for comparison, it seems reasonable to hypothesize that toxicity of the higher brominated congeners would be lower than that of the lower brominated congeners, given the lower observed bioaccumulation, lower absorption and lower water solubility of the former. The Great Lakes Water Quality Initiative (U.S. EPA 1995) calls for regulation of the discharge of bioaccumulating compounds. Calculation of surface water quality criteria for the protection of fish and aquatic life requires toxicity data for at least one species in each of eight families. In Wisconsin, at a minimum, there must be toxicity data available for one of three cladoceran species (*Ceriodaphnia sp.*, *Daphnia sp.*, or *Simocephalus sp.*) to even establish a secondary (or Tier II) value (Chapter NR 105, Wisconsin Administrative Code). Given the bioaccumulative and ubiquitous nature of BDE-47, it is especially important that such basic aquatic toxicity data be established for this PBDE congener.

As noted above, PBDEs are bioaccumulating in organisms throughout the Great Lakes region; however, little is known regarding specific sources of PBDEs to the Great Lakes. Studies are currently being conducted to examine the history of input and dominant methods of transport of PBDEs to Lake Michigan (W.Sonzogni et al., University of Wisconsin Sea Grant Institute

Grant). More detailed sampling of a particular tributary would complement these studies and would provide more specific information on sources of discharge. Concentrations of PBDEs in sediment have been shown to increase downstream of some industries (Sellstrom et al. 1998). In addition, high concentrations of PBDEs have been measured in sewage sludge (Hale et al. 2001) and in organisms placed in a sewage treatment plant pond (Rimkus and Wolf 2001); however, at the time the present studies were initiated, concentrations of PBDEs in industrial and sewage treatment plant effluents had not been examined.

Project Objectives and Hypotheses

The purpose of this project was to address, in part, each of the major data gaps identified above: estrogenic activity, aquatic toxicity, and sources of PBDEs. An initial objective was to establish standard operating procedures (SOPs) for the analysis of PBDEs in water, wastewater effluent, and sediment. While outside of the main objectives of the project, this initial work was integral to the completion of the main objectives.

Specific research objectives and null hypotheses follow.

Objective 1: Determine the estrogenic activity of BDE-47 and BDE-99 using the E-screen assay.

Ho_1: Breast cancer cell proliferation does not differ significantly among treatments of BDE-47 (including the control).

Ho_2: Breast cancer cell proliferation does not differ significantly among treatments of BDE-99 (including the control).

Objective 2: Determine the acute and chronic toxicity of BDE-47 to the cladoceran, *Ceriodaphnia dubia*.

Ho_1: Mean mortality of *Ceriodaphnia dubia* does not significantly differ among BDE-47 treatments (including the control).

Ho_2: Mean reproduction of *Ceriodaphnia dubia* does not significantly differ among BDE-47 treatments (including the control).

Sub-objectives:

a) Calculate an EC_{50} (effective concentration for 50% of the population) value for mortality in the acute toxicity test.
b) Calculate an IC_{25} value (inhibition concentration for 25% of the population) for reproduction in the chronic toxicity test.
c) Calculate NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values for reproduction in the chronic toxicity test.

Objective 3: Determine the concentration of PBDEs in sediments of the Sheboygan River basin

in relation to specific effluent outfalls.

Given limited funds, the number of sample sites and the number of replicate samples was low. Therefore, the objective here was to conduct an initial reconnaissance-type evaluation of PBDE contamination in sediments.

Objective 4: Determine the concentration of PBDEs in wastewater treatment plant and industrial effluents in the Sheboygan River basin.

Given limited funds, the number of sample sites and the number of replicate samples was low. Therefore, the objective here was to conduct an initial reconnaissance-type evaluation of PBDE contamination in effluents.

Specific congeners measured for each project objective can be found in Table 1, and the CAS registration numbers for each of these congeners can be found in Table 2.

METHODS

Objective 1- Estrogenic Activity of BDE-47 and BDE-99

The E-screen assay was used to determine if PBDE congeners BDE-47 and BDE-99 exhibit estrogenic activity. The E-screen assay utilizes the MCF-7 breast cancer cell line. These cells proliferate in the presence of estrogen or estrogen-like compounds.

Cell Culture. MCF-7 cells were obtained from Drs. C. Sonnenschein and A.M. Soto (Tufts University School of Medicine, Boston, MA). MCF-7 cells were cultured in Dulbecco's modified Eagle Medium (ICN Biomedicals, Aurora, OH) supplemented with 5% fetal bovine serum (FBS) growth medium (Hyclone Laboratories, Logan UT) at 37^{o}C and 6.5% CO_2 in 75 cm^2 tissue culture flasks. Media was changed every two to three days, and the cells were subcultured every seven days. Stocks of MCF-7 cells were stored in liquid nitrogen and new cells were thawed to replace cells that had undergone approximately 20 passages of subculturing.

E-Screen Assay. Seven days after being subcultured, cells were trypsinized and counted on an EPICS XL flow cytometer (Coulter Corporation, Miami, FL). Cell counts were conducted using FlowCount beads (Coulter Corporation, Miami, FL), an FDA-approved microbead standard originally developed for enumeration of blood cells in human patients. Once the concentration of cells was determined, cells were seeded in 24-well plates at 20,000 to 30,000 cells per well, in 1 mL of culture media. Twenty-four hours after seeding, the culture media was removed and experimental media was added.

The experimental media was phenol red-free Dulbecco's modified Eagle Medium (Irvine Scientific, Irvine, CA) supplemented with 5% FBS that had been stripped of steroids with charcoal dextran following a procedure described by Payne et al. (2000). Briefly, the FBS was incubated with activated charcoal and dextran and then centrifuged and filtered (0.2 μm) to remove the charcoal. This experimental media is referred to as CD-media.

To obtain a standard curve for comparisons, MCF-7 cells were exposed to15 different treatments (*n*=4; with concentrations ranging from 0.1 to 10,000 pM) of 17β-estradiol (Sigma Chemical, St. Louis, MO) dissolved in ethanol in CD-media. Three plates were needed to obtain a complete standard curve, and four control wells (no 17β-estradiol and no PBDEs) were included on each plate.

BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) and BDE-99 (2,2',4,4',5-pentabromodiphenyl ether) analytical standards (in nonane) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA) at a concentration of 50 μg/mL for use in the E-screen assays. Based on the results of initial range-finding assays, 200 μL of the BDE-47 standard was concentrated under a gentle stream of nitrogen to 100 μL. A 100-fold dilution was made in CD-media to give a concentration of 2.0×10^{-6} M BDE-47. A 1.5-fold dilution series was then made in CD-media to give five additional concentrations of BDE-47. One mL of the BDE-99 standard was concentrated under a gentle stream of nitrogen to 50 μL. A 120-fold dilution was made in CD-media to give a concentration of 1.48×10^{-5} M BDE-99. A 2-fold dilution series was then made in CD-media to give five additional concentrations of BDE-99. Each concentration, or treatment, of BDE-47 or BDE-99 was applied to six wells at a volume of 0.5 mL each. Two plates were needed to obtain a complete PBDE (BDE-47 or BDE-99) curve, and four control wells (no 17β-estradiol and no PBDEs) were included on each plate.

After five days of incubation, cell proliferation measured using a sulphorhodamine B (SRB) protein assay (Sigma Chemical, St. Louis, MO). The CD-media was removed from the cells and 10% trichloroacetic acid (TCA) solution was added to each well to fix the cells. After 20 to 30 minutes, the TCA was removed, the wells were allowed to dry and the SRB solution (0.4% SRB dye in a solution of 1% acetic acid and 99% distilled water) was added to each well to stain the cells. After 20 to 30 minutes, the residual SRB dye was removed by rinsing with 1% acetic acid. The remaining dye was redissolved using 10 mM Tris solution (pH 10.5) and absorbance was read at a wavelength of 515 nm on a Molecular Devices microplate reader (Sunnyvale, CA).

Cytotoxicity. As a positive control, and to determine the toxicity of each PBDE congener to the MCF-7 cells, 17β-estradiol was added to two of the six wells for each PBDE treatment, at a concentration of 100 pM.

Data Analysis. Maximum cell proliferation of MCF-7 cells exposed to 17β-estradiol was set as 100%. To determine the concentration of 17β-estradiol that caused 50% of the maximum proliferation (EC_{50}), the standard curve was fit with a four-parameter logistic equation using Softmax PRO v. 2.6 analytical software, the software associated with the microplate reader. Cell proliferation of MCF-7 cells exposed to BDE-47 and BDE-99 was expressed as a % of the maximum response relative to the positive controls (17β-estradiol spikes) and negative controls (no 17β-estradiol and no PBDEs) on the PBDE plates as follows:

$$\% \text{ maximum response} = (Abs_{sample} - Abs_{blank}) / (Abs_{17\beta\text{-estradiol spike}} - Abs_{blank}) * 100$$

where Abs means absorbance. To determine if either of the PBDEs had a significant effect on cell proliferation, results (absorbance and % maximum proliferation) were analyzed using

analysis of variance (ANOVA) followed by Student-Newman-Kuhls multiple comparison procedure (SAS v. 8.2, SAS Institute, 2001) where ANOVA F-tests were significant ($p<0.05$).

Objective 2- Toxicity of BDE-47 to *Ceriodaphnia dubia*

To test the acute and chronic toxicity of BDE-47 on *Ceriodaphnia dubia*, a series of laboratory toxicity tests were conducted. Acute toxicity tests followed ASTM standard method E 729-96, with the exception that there were four replicates per treatment instead of three, and chronic toxicity tests followed ASTM standard method E 1295-89 (re-approved in 1995).

Range-Finding Acute Toxicity Test. A 48-hour, static renewal, range-finding acute toxicity test was conducted with *Ceriodaphnia dubia* that were less than 24-hours old. Five *C. dubia* were randomly allocated to each of four replicate 30 mL glass beakers per treatment or control. Because the solubility of BDE-47 is low (approximately 15 µg/L), it was necessary to use dimethylsulfoxide (DMSO) to obtain higher concentrations of BDE-47 in the test water. DMSO exhibits low toxicity in Daphnid species (e.g., LC_{50} of 58,200,000 µg/L for *Daphnia magna*; ECOTOX database, U.S. EPA 2003). Treatments consisted of the following (nominal) concentrations: 0, 0 (+DMSO; carrier control), 3.89, 6.48, 10.8, 18, 30, and 50 µg BDE-47/L. Concentrations in this range-finding acute toxicity test were chosen based on published data for another daphnid species, *Daphnia magna* (LC_{50} of 14 µg/L for the commercial pentaBDE product; in Hardy 2002). *Ceriodaphnia dubia* is frequently more sensitive to organic contaminants than *Daphnia magna*. A 50 µg BDE-47/L stock solution was prepared by placing 1.0 mL of the 50 µg BDE-47/mL analytical standard (in nonane; Cambridge Isotopes Laboratories, Inc., Andover, MA) in a 5 mL amber glass vial, evaporating off the nonane under a gentle stream of nitrogen in a fume hood, adding 0.5 mL DMSO to the vial, rinsing the vial five times with dilution water into a 1 L volumetric flask, and then filling the volumetric flask to the line with dilution water. The dilution water was reconstituted moderately hard water. A portion of this stock solution was used as the highest treatment. Additional treatments were prepared through serial (60%) dilution. All glassware (including test chambers) had been washed and rinsed prior to use, according to the standard operating procedure for cleaning glassware contained in Appendix A.

Beakers were kept in an incubator set at 25^{o} C, with a 16-hour light and 8-hour dark photoperiod. Organisms were not fed during the range-finding acute toxicity test, but were checked daily for mortality or immobilization. Treatments were renewed at 24 hours. Hardness and alkalinity were measured in the dilution water at the start of the test. Dissolved oxygen, pH, and conductivity were measured in each treatment at 0, 24 [in both final (before renewal) and initial (after renewal) solutions], and 48 hours. Temperature was measured in beakers in five locations (the four corners and the middle of the board) daily. Concentrations of BDE-47 were not measured.

Toxicity of DMSO. Because high (30%) mortality was observed in the carrier control treatment in the range-finding acute toxicity test, an acute toxicity test was conducted to determine if DMSO is toxic to *C. dubia*. Five *C. dubia* were randomly allocated to each of four replicate plastic beakers per treatment or control. There were seven treatments: 0 (moderately hard water

only), 0.0315, 0.063, 0.125, 0.25, 0.5, and 1.0 mL/L DMSO. Organisms were exposed for 48 hours and were checked daily for mortality or immobility.

First "Definitive" Acute Toxicity Test. A 48-hour definitive acute toxicity test was conducted as described above for the range-finding acute toxicity test, with the following exception: treatments consisted of (nominal) concentrations of 0, 0 (+DMSO; carrier control), 2.33, 3.89, 6.48, 10.8, 18, 30, and 50 µg BDE-47/L.

Plastic Vs. Glass, Covered Vs. Uncovered. Because high (50 to 100%) mortality was observed in all treatments and controls in the first definitive acute toxicity test, additional toxicity tests were conducted to determine if this mortality had something to do with the beakers being used in the tests. These toxicity tests were also designed to determine if volatility of BDE-47 may affect results. Acute toxicity tests were conducted in four different types of beakers: covered glass beakers, uncovered glass beakers, covered plastic beakers, and uncovered plastic beakers. Glass covers were placed on glass beakers, and plastic on plastic. Five *C. dubia* were randomly allocated to each of one beaker per BDE-47 treatment or two beakers per control, in each of the four different beaker-type tests. Treatments consisted of 0, 0 (+DMSO), 1.58, 3.2, 6.3, 12.5, 25, and 50 µg BDE-47/L in each of the four different beaker-type tests. The four toxicity tests were conducted simultaneously. Organisms were exposed for 48 hours and were checked daily for mortality or immobility.

Comparison of Different Glassware Washing Procedures. Based on the results of the plastic versus glass toxicity test, it was determined that something must be adhered to the glass beakers (most of which were new) that is toxic to *C. dubia*, and that is not removed when washed and rinsed according to the standard operating procedure for cleaning glassware (Appendix A). To determine how best to wash the glassware to ensure optimum control survival, acute toxicity tests were conducted to compare survival in control waters (moderately hard water only and moderately hard water plus DMSO) held in chambers washed in one of the following four ways: (1) not washed (disposable plastic beakers), (2) washed following standard operating procedure (SOP) for washing glassware (glass beakers), (3) washed following SOP with additional acetone rinse, or (4) acid soaked prior to being washed following SOP and acetone rinsed. Wash treatment (1) (not washed) is typically used in whole effluent toxicity (WET) testing when plastic beakers are not washed nor rinsed prior to use. Glass beakers washed according to (2) were hand washed with tap water, rinsed with tap water, rinsed with 10% hydrochloric acid, rinsed with tap water, rinsed with acetone, and then rinsed three times with reverse osmosis water. Glass beakers washed according to (3) were hand washed with tap water, rinsed with tap water, rinsed with 10% hydrochloric acid, rinsed with tap water, rinsed with acetone, rinsed three times with reverse osmosis water, rinsed a second time with acetone, and then rinsed three times with Type I water. Glass beakers washed according to (4) were washed in the Standard Cycle (see Appendix A), rinsed with reverse osmosis water, soaked in trace-metal clean 20% nitric acid for 24 hours, rinsed three times with reverse osmosis water, rinsed with acetone, and then rinsed three times with reverse osmosis water.

Second Definitive Acute Toxicity Test. A second 48-hour definitive acute toxicity test was conducted as described above for the first definitive acute toxicity test, with the following exception: concentration of BDE-47 was measured in all treatments at 0 hour (initial), and in all

but the 30 and 50 µg/L treatments at 48 hours (final). Initial samples (*n*=1;100 mL) were collected from stock solutions for each treatment, and final samples (*n*=1; approximately 75 mL) were collected by compositing the solution in each of the four replicate beakers for each treatment. Samples were collected in amber glass bottles with Teflon-lined caps and stored at 4°C for up to two weeks until extracted. Sample bottles had been washed and rinsed prior to use, according to the standard operating procedure for cleaning glassware contained in Appendix A. Test chambers (glass beakers) had been washed and rinsed prior to use, according to the revised standard operating procedure for cleaning glassware also contained in Appendix B (which includes an additional step of soaking for 24 hours in 20% hydrochloric acid).

Third Definitive Acute Toxicity Test. Because we observed a significant decrease in concentrations over a 24 hour period in the second definitive acute toxicity test, and because final concentrations were not measured for the two highest treatments in that test, a third 48-hour definitive acute toxicity test was conducted as described above for the second definitive acute toxicity test, with the following exception: concentration of BDE-47 was measured in each treatment at 0 hour (initial), 24 hours (final), 24 hours (initial), and 48 hours (final). Again, initial samples (*n*=1;100 mL) were collected from stock solutions for each treatment, and final samples (*n*=1; approximately 75 mL) were collected by compositing the solution in each of the four replicate beakers for each treatment.

Chronic Toxicity Test. A 7-day, three-brood renewal toxicity test was conducted to test the effects of low concentrations of BDE-47 on survival and reproduction in *C. dubia*. One *C. dubia* (less than 12 hours old) was randomly allocated to each of ten individual replicate glass beakers per treatment. Treatments consisted of the following (nominal) concentrations: 0, 0 (+DMSO; carrier control), 1.9, 3.2, 5.4, 9.0, and 15 µg BDE-47/L (60% dilution series). A 25 µg BDE-47/L stock solution was prepared by placing 1.0 mL of the 50 µg BDE-47/mL analytical standard (in nonane) in a 5 mL amber glass vial, evaporating off the nonane under a gentle stream of nitrogen in a fume hood, adding 0.5 mL DMSO to the vial, rinsing the vial five times with dilution water into a 2 L volumetric flask, and then filling the volumetric flask to the line with dilution water. The dilution water was reconstituted moderately hard water. Treatments were prepared through serial (60%) dilution. All glassware, with the exception of beakers used as test chambers, had been washed and rinsed prior to use, according to the standard operating procedure for cleaning glassware contained in Appendix A. Glass beakers used as test chambers had been washed and rinsed prior to use, according to the revised standard operating procedure for cleaning glassware contained in Appendix B (which includes an additional step of soaking for 24 hours in 20% hydrochloric acid). Beakers were kept in an incubator set at 25°C, with a 16-hour light and 8-hour dark photoperiod. Organisms were fed *Raphidocelis subcapitata* algae and YTC (yeast trout chow) during the test. Test solutions were renewed daily. At time of renewal, first-generation *C. dubia* were checked and recorded as alive or dead. Any live or dead offspring were counted, recorded and discarded. Live first-generation *C. dubia* were transferred to a clean chamber containing new test solution.

Hardness and alkalinity were measured in the dilution water at the start of the toxicity test. Dissolved oxygen, pH, and conductivity were measured in each treatment on daily [in both final (before renewal) and initial (after renewal) solutions, where appropriate]. Temperature was measured in beakers in five locations (the four corners and the middle of the board) daily.

Concentrations of BDE-47 were measured in all treatments on days 0 (initial), 1 (final), 6 (initial) and 7 (final). Initial samples (n=1;100 mL) were collected from stock solutions for each treatment, and final samples (n=1; approximately 75 mL) were collected by compositing the solution in each of four (randomly selected) replicate beakers for each treatment. Samples were collected in amber glass bottles with Teflon-lined caps and stored at 4^{o}C for up to two weeks until extracted.

Data Analysis. ANOVA was used to compare mortality (in the range-finding acute, the second and third definitive acute, and in the chronic toxicity tests) and reproduction (in the chronic toxicity test) among treatments. Mortality data were arcsine square-root transformed prior to analysis. Assumptions of ANOVA were tested, and reproduction data were square-root transformed to satisfy assumptions of heterogeneity of variance. Where ANOVA results indicated a significant difference (p<0.05) among treatments, the Ryan-Einot-Gabriel-Welsch multiple range test was applied to determine which treatments were significantly different from each other (SAS v. 8.2, SAS Institute, 2001). A 48-hour LC_{50} (lethal concentration for 50% of the population) value was calculated for the second and third definitive acute toxicity tests using the trimmed Spearman-Karber method (Hamilton et al. 1977). An IC_{25} (inhibition concentration for 25% of the population) value was calculated for reproduction in the chronic toxicity test using U.S. EPA's IC_p method (U.S. EPA 1993). A lowest observed effect concentration (LOEC) was identified in the chronic toxicity test as the lowest treatment concentration with reproduction significantly different from that of the controls. A no observed effect concentration (NOEC) was identified in the chronic toxicity test as the highest treatment concentration with reproduction not significantly different from that of the controls. A maximum acceptable toxicant concentration (MATC) value was calculated as the geometric mean of the NOEC and the LOEC.

Objectives 3 and 4- Concentrations of PBDEs in Effluents and Sediments

Location of Sample Sites. To examine possible sources of PBDEs to Lake Michigan, effluents and sediments were sampled from the Sheboygan River basin. Effluent samples were collected at select facilities located on the Mullet and Sheboygan Rivers, and sediments were collected upstream and downstream of those facilities. Land use in the Mullet River (tributary to the Sheboygan River) is largely agricultural, while land use in the lower Sheboygan River watershed is mixed, with a large amount of industry. This industry includes plastics and furniture manufacturing— two industries that are known to use PBDEs. The lower 14-mile stretch of the Sheboygan River, including the Inner Harbor, is highly contaminated with PCBs and heavy metals and was added to the National Priorities List (and designated a Superfund site) in 1986. This area was also designated an Area of Concern (AOC) by the U.S. EPA.

Two municipal wastewater treatment plants (WWTPs) and three industries were chosen as sample locations (Figure 1). WWTP A is located on the Mullet River and receives primarily domestic waste, while WWTP B is located on the Sheboygan River and receives both domestic and industrial waste. Both of these plants use activated sludge systems with phosphorus removal. Industry A is located on the Mullet River and produces cheeses. Industry B is located on the Sheboygan River and produces various plastic products, including casual furniture, health care products and humidifiers. Industry C is also located on the Sheboygan River and produces water filtration products.

Geolocation of Sample Sites. Each sediment and effluent sampling site was geolocated (latitude and longitude) using a Trimble Pro-XRS global positioning system (GPS) unit (Trimble Navigation Limited, Sunnyvale, CA). Locational data were collected in the Wisconsin Transverse Mercator (WTM) format with sub-meter accuracy. Data were stored in the datalogger and downloaded onto a computer using Pathfinder software. Quality control was determined by the datalogger. It communicates with the GPS receiver to set specific GPS parameters required for optimal accuracy. Data validity is determined by the number of satellites. If there are too few satellites, a warning tone will sound to identify the data. The same validity checks are built into the Pathfinder software.

Collection of Effluent Samples. At each facility, two 1-L effluent grab samples were collected at a point as near to the final outfall as possible. One effluent blank sample (Milli-Q water) was also collected at each facility. Powder-free latex gloves were worn at all times while handling the effluent. Samples were collected in 2-L amber glass bottles with Teflon-lined lids. Sample containers and any sampling equipment used (e.g., glass funnel) had been washed and rinsed in acetone prior to use according to the methods contained in Appendix A. Sample containers were labeled in black indelible ink with the sampling location, sample type (effluent), date, sampler's initials, the requested analysis (PBDEs), and a unique sample number. Samples were preserved by keeping them on ice and then by storing them at 4^{o}C in the laboratory until they were extracted prior to analysis. Temperature, pH, conductivity, hardness, and dissolved oxygen were measured in effluent at each facility at the time of effluent collection. Digital photographs were also taken at each facility.

Collection of Sediment Samples. Sediment was collected upstream and downstream of each facility where effluent was collected (recognizing that such samples are not independent and that PBDEs may migrate through sediment transport), with the exception that no sediment was collected downstream of WWTP B because the downstream site is located out in Lake Michigan. Also, sediment was collected at two sites upstream of Industry C, designated “upstream- far” and “upstream- near”. Sample sites were accessible from the shoreline and were located in sediment depositional zones. At each sample location, two composite sediment samples were collected from the top 5 to 10 cm of substrate using a stainless steel corer or Petit Ponar dredge, a stainless steel mixing bowl and stainless steel spoon. Two sediment blank samples were collected at each of two locations: the downstream location for Industry A on the Mullet River, and the downstream location for Industry B on the Sheboygan River. Blank sediment samples were collected by running quartz sand (catalog #S25-500, Fisher Scientific, Hanover Park, IL) through the sampling equipment. Sampling equipment was cleaned with a detergent solution and rinsed with Milli-Q water after each sample was collected. Powder-free latex gloves were worn at all times while handling the sediment. Samples were transferred to 1-L amber glass jars with Teflon-lined lids, and stored on ice. Sample containers and sampling equipment had been washed and rinsed in acetone prior to use according to the methods contained in Appendix A. Sample containers were labeled in black indelible ink with the sampling location, sample type (sediment), date, sampler's initials, the requested analysis (PBDEs), and a unique sample number. Samples were preserved by keeping them on ice and then by storing them at 4^{o}C in the laboratory until they were extracted prior to analysis. River water temperature, pH, conductivity,

total dissolved solids, water hardness, and dissolved oxygen were measured at each sample site at the time of sediment collection. Digital photographs were also taken at each sample site.

Data Analysis. Mean (and standard deviation) concentrations of each PBDE congener were calculated for replicate sediment and effluent samples collected at each site. Because sample size was low, it was not possible to statistically compare PBDE concentrations among locations.

Analysis of PBDEs

Prior to collecting and processing any samples, test effluent and blank samples were collected and analyzed to determine whether contamination may be a problem, and if so, to identify and eliminate to the extent possible, any sources of contamination. Results of these initial analyses indicated contamination was not a problem, and that PBDEs were detectable in effluents in the low ng/L-range.

All water (toxicity test solution), effluent and sediment samples were stored in a refrigerator at $4^{o}C$ until they were extracted and analyzed. The holding time was less than seven days before extraction for water and effluent samples, and less than 14 days before extraction for sediment samples. Holding time for all extracted samples was less than 40 days.

Standard operating procedures (SOPs), established as an initial objective of this project, were used in the analysis of PBDEs in water, sediment, and effluent (Appendices C, D, and E). Briefly, water samples collected in the toxicity tests were solvent extracted with methylene chloride using a separatory funnel. Extracts were concentrated under a stream of nitrogen, transferred to iso-octane, and treated with concentrated sulfuric acid. After dilution to an appropriate volume, extracts were injected on a 1995 Hewlett Packard model 5890 Series II Plus gas chromatograph equipped with an electron capture detector and analyzed for BDE-47. Analysis was performed with a 60 M DB-5 column, 0.25 mm OD, 0.10 u film.

Effluent samples were solvent extracted with methylene chloride using a separatory funnel. Extracts were concentrated on a rotoevaporator to approximately 5 ml. Two milliliters of iso-octane was added to each and the volume was reduced under a stream of nitrogen. After Florisil and/or silica gel clean-up, extracts were injected on the gas chromatograph equipped with an electron capture detector and analyzed for BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154. PBDE congeners 183 and 209 were not measured because they do not elute within the 101-minute run that was used to measure the other congeners, and would have required a separate run (which would have incurred additional costs).

Sediment samples were air dried and homogenized by sieving. Samples were then Soxhlet extracted with hexane/acetone for sixteen hours. After concentrating with a roto-evaporator the extracts were run through a column containing Florisil. The first fraction of each was concentrated and run through a column containing silica-gel. Final extracts were concentrated and injected onto a gas chromatograph equipped with an electron-capture detector and analyzed for BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154.

Pure analytical PBDE standards were used to determine % recoveries for each PBDE congener analyzed. Because the National Institute of Standards and Technology (NIST) does not have standards available yet for PBDEs in various matrices, performance evaluation samples of this nature were not analyzed. However, several quality controls for PBDE analysis were incorporated, including analysis of field blanks, method blanks, duplicate samples, calibration check standards, matrix spikes, internal standards, and surrogate standards.

Blanks were used to check for contamination throughout the collection and analytical processes. PBDE-free water was extracted as a water and effluent blank, and clean quartz sand was extracted as a sediment blank. Method blanks were analyzed once for each batch of samples (<20 samples) analyzed. Blanks were deemed acceptable if concentrations of the target analytes were below the limit of quantitation. Duplicate samples were used to evaluate precision of analysis. Duplicate samples were deemed acceptable if the relative percent difference was less than 50%. A calibration check standard is a solution that contains the PBDE congeners of interest at concentrations in the middle of the calibration range. Calibration check standards were used to determine if the instrument was responding to within 25% of the initial calibration. They were analyzed at the midpoint and end of each batch of samples run. Matrix spikes were made up in clean water (for water and effluent samples) and clean quartz sand (for sediment samples) as additional quality control checks. Matrix spikes were run for each batch of samples. Matrix spike samples were deemed acceptable within a range of 50 to 135% (based on previous SLOH experience with analysis of serum samples for PBDEs). A surrogate standard was used to monitor analytical recovery. PCB congener 166 was added to all samples prior to the initial sample extraction step to cover the whole analytical process. This PCB congener is not commonly found in the environment and has a similar structure to many PBDE congeners. PCB congener 204 was used as an internal standard. It was spiked into samples just prior to GC analysis and used as a retention time reference peak and internal standard for quantitation.

RESULTS

Objective 1- Estrogenic Activity of BDE-47 and BDE-99

Exposure of MCF-7 cells to BDE-47 and to BDE-99 in E-screen assays resulted in significant proliferation; however, the level of proliferation was much lower than that of cells exposed to17β-estradiol. Maximum cell proliferation of 17β-estradiol was set as 100%, and maximum proliferation of BDE-47 and BDE-99 was about 10% of that of 17β-estradiol. Cell proliferation response to 17β-estradiol was sigmoidal, with an EC_{50} (effective concentration for 50% of the cells) of 3.7 pM (Figure 2).

To determine the toxicity of BDE-47 and BDE-99 to the MCF-7 cells, 17β-estradiol was added to two of the six wells for each of the seven PBDE treatments. Cell proliferation in the highest two treatments of both BDE-47 and BDE-99 was significantly decreased relative to the control and to four lower treatments ($p<0.05$, Student-Newman Kuhls), indicating that each of these congeners is toxic to MCF-7 cells at higher concentrations (Figures 3 and 4).

Maximum cell proliferation for BDE-47 and BDE-99 occurred at concentrations of 5.93E-07 M (or 288 μg/L BDE-47) and 3.69E-06 M (or 2,084 μg/L BDE-99), respectively (Figures 5 and 6). It was not possible to determine the effective concentration for 50% of the population of cells (EC_{50}) for either congener because both were cytotoxic at the upper range of concentrations tested.

Objective 2- Toxicity of BDE-47 to *Ceriodaphnia dubia*

Results of the range-finding acute toxicity test indicated that BDE-47 was toxic to *C. dubia* and that an appropriate range of concentrations had been selected for the test. However, mortality in the carrier (DMSO) control was too high (30%) for the test to be valid (Figure 7). To determine if the DMSO was toxic, a test was conducted that examined the toxicity of seven different concentrations of DMSO (ranging from 0 to 1 mL DMSO/L) to *C. dubia*. Mortality ranged from 0 to only 5% (± standard error of 5%), indicating DMSO was not toxic (data not shown). So a first "definitive" acute toxicity test was conducted with BDE-47. Mortality in this test ranged from 50 to 100%, with 50 and 75% mortality in the control and carrier control, respectively (Figure 8). To determine the cause of such high control mortality, two additional sets of toxicity tests were conducted.

The first set of tests compared the toxicity of BDE-47 to *C. dubia* in uncovered plastic, covered plastic, uncovered glass, and covered glass beakers. Mortality was much higher in the glass beakers than in the plastic beakers containing BDE-47, as one would expect given that BDE-47 is an organic chemical. And, as expected, there was little difference in mortality between covered and uncovered beakers of a given type, indicating volatility was not a concern. However, high control mortality (60 to 90%) was observed for *C. dubia* in glass beakers and no control mortality was observed for those in plastic (Figure 9). The second set of tests compared mortality of *C. dubia* held in control waters in beakers washed one of four ways as described in the methods section. Mortality in these tests ranged from 0% in the wash treatment that included an acid soak to 90% in the wash treatment that followed the SOP (Figure 10). As a result of these tests, the SOP for washing glassware was revised to include a 24-hour soak in 20% HCl. Glass beakers used in all acute and chronic toxicity tests that followed were washed according to the revised SOP.

In the second definitive acute toxicity test, control mortality was low (5%) in both the control and carrier control treatments. There were significant differences in mortality among treatments, and mortality ranged from 5 to 100% (Figure 11). The calculated LC_{50} value was 4.60 μg/L, with a 95% confidence interval of 4.12 to 5.14 μg/L, based on measured concentrations. Concentrations of BDE-47 decreased an average of 37% over the course of 48 hours. (Solutions were renewed at 24 hours, but with the same stock solutions made up at 0 hours.) All organisms in the two highest treatments (nominal concentrations of 30 and 50 μg/L) died within 24 hours; therefore, these two treatments were not renewed at 24 hours. This meant there were no 48-hour final concentration measurements for these two treatments. Given the significant decrease in concentration over time, 48-hour final concentrations were estimated for the two highest treatments, so that average concentrations could be calculated and used in statistical and other analyses. Final 48-hour concentrations were estimated by subtracting the average % decrease for all other treatments (37%) from the 0 hour concentration for each of the two highest treatments.

Nominal concentrations ranged from 0 to 50 µg BDE-47/L, and average measured concentrations (including those estimated for the two highest treatments) ranged from below detection to 28.7 µg BDE-47/L. The limit of detection, based on instrument sensitivity was estimated at 0.05 µg/L for 70 mL samples, and 0.035 µg/L for 100 mL samples. All samples were at least 70 mL in volume. Calibration standards were run at 2.0, 5.0, 10, 20, 30, and 40 µg/L. A quantitation limit based on the lowest standard was 0.14 µg/L for 70 mL samples and 0.10 µg/L for 100 mL samples. Percent recovery of the surrogate (PCB-166) ranged from 86.7 to 99.1%. Percent recovery of the spike (BDE-47) ranged from 96.6 to 100%. Concentrations of BDE-47 in all method blanks were below detection. Temperature in the second definitive acute toxicity test averaged 25.0^{o}C, and dissolved oxygen, pH, and conductivity ranged from 8.1 to 8.2, 7.7 to 8.3, and 281 to 309, respectively (Table 3).

In the third definitive acute toxicity test, control mortality was low (5%) in both the control and carrier control treatments. There were significant differences in mortality among treatments, and mortality ranged from 5 to 100% (Figure 12). The calculated LC_{50} value for this test was 2.95 µg/L, with a 95% confidence interval of 2.39 to 3.64 µg/L, based on actual measured concentrations. Concentrations of BDE-47 decreased an average of 26% over the course of 24 hours. (Solutions were renewed at 24 hours with new stock solutions in this test.) Nominal concentrations ranged from 0 to 50 µg BDE-47/L, and average measured concentrations ranged from 0.01 to 34.5 µg BDE-47/L. The limit of detection, based on instrument sensitivity was estimated at 0.05 µg/L for 70 mL samples and 0.035 µg/L for 100 mL samples. All samples were at least 70 mL in volume. Calibration standards were run at 2.0, 5.0, 10, 16, 20, and 30 µg/L. A quantitation limit based on the lowest standard was 0.14 µg/L for 70 mL samples and 0.10 µg/L for 100 mL samples. Percent recovery of the surrogate (PCB-166) ranged from 94.4 to 108%. Percent recovery of the spike (BDE-47) ranged from 98.4 to 102%. Concentrations of BDE-47 in all method blanks were below detection. Temperature in the third definitive acute toxicity test averaged 24.6^{o}C, and dissolved oxygen, pH, and conductivity ranged from 8.2 to 8.6, 8.0 to 8.3, and 243 to 269, respectively (Table 4).

In the chronic toxicity test, mortality ranged from 0 to 90%, with 0 and 10% mortality in the control and carrier control treatments, respectively. There were significant differences in mortality among treatments, with treatments (measured concentrations) 3.3, 5.3, and 7.9 µg BDE-47/L significantly different from the control (p<0.05, Ryan-Einot-Gabriel-Welsch multiple range test; Figure 13). Reproduction was significantly affected by BDE-47, and mean number of neonates ranged from 0 to 35.2 (Figure 14). However, very low or no reproduction in the highest three treatments was influenced by high mortality. Therefore, only those treatments with mortality not significantly different from the control were included in the analysis of variance (ANOVA). Results of ANOVA indicated reproduction was significantly lower in the highest treatment not significantly different from the control: 2.0 µg/L. This was the LOEC, and the NOEC was 1.4 µg/L. The calculated MATC was 1.66 µg/L with a standard deviation of 0.42 µg/L. The calculated IC_{25} value was 2.05 µg BDE-47/L, with a 95% confidence interval of 1.86 to 2.21 µg BDE-47/L. The limit of detection, based on instrument sensitivity was estimated at 0.035 µg/L. Calibration standards were run at 2.0, 5.0, 10, 16, 20, and 30 µg/L. A quantitation limit based on the lowest standard was 0.10 µg/L. Percent recovery of the surrogate (PCB-166) ranged from 94 to 100%. Percent recovery of the spike (BDE-47) ranged from 89 to 94%.

Concentrations of BDE-47 in all method blanks were below detection. Temperature in the chronic test averaged 25.1°C, and dissolved oxygen, pH, and conductivity ranged from 8.5 to 8.6, 8.3 to 8.5, and 283 to 315, respectively (Table 5).

Objectives 3 and 4- Concentrations of PBDEs in Effluents and Sediments

Effluents were collected at five facilities, and sediments were collected at 10 locations in the Sheboygan River basin. Longitude and latitude data associated with each of these locations is presented in Table 6. Date and time of collection of effluent and sediment samples, and associated water chemistry data are presented in Table 7.

Only two PBDE congeners were detected in effluents collected from facilities located in the Sheboygan River basin: BDE-47 and BDE-99. Both of these congeners were detected in effluents collected from WWTP B and Industry C, while only BDE-99 was detected in effluents collected from WWTP A (Table 8). No PBDEs were detected in effluents collected from Industry A or Industry B. Concentrations ranged from below detection to 4.5 and 3.3 ng/L, for BDE-47 and BDE-99, respectively. Concentrations of BDE-47 were slightly higher than those of BDE-99 in each effluent sample where both were detected. The report limit for all PBDE congeners, with the exception of BDE-47, was 1.0 ng/L. The report limit for BDE-47 was 2.0 ng/L. Concentrations of all nine PBDE congeners analyzed were less than their report limits in all effluent blanks.

Six different PBDE congeners were detected in sediment in the Sheboygan River basin, with concentrations ranging from 0.45 to 6.0 ng/g (Table 9). The report limit for all PBDE congeners in sediment was 0.4 ng/g. Concentrations of PBDEs in all field blank samples (quartz sand) were below the report limits for all congeners, with the exception of one of the blank samples collected downstream of Industry A on the Mullet River, in which concentrations of BDE-47, BDE-99, and BDE-100 were 1.6, 2.7, and 0.42 ng/g, respectively. BDE-47 and BDE-99 were the congeners detected most frequently, and concentrations of BDE-99 were always slightly higher than those of BDE-47 in each sediment sample where both were detected. BDE-28, BDE-67, and BDE-138 were never detected. Sediment that contained the highest number of PBDE congeners, and the highest concentrations of individual PBDE congeners was collected at the site located just upstream ("upstream-near") of Industry C on the lower Sheboygan River. Average concentrations were 3.8, 0.58, 6.0, 1.4, 0.84, and 0.61 ng/g for BDE-47, BDE-85, BDE-99, BDE-100, BDE-153, and BDE-154 at this location. No PBDEs were detected in sediments collected just upstream or downstream of Industry A or Industry B.

DISCUSSION

This study showed that PBDE congeners BDE-47 and BDE-99 can act as estrogen receptor agonists *in vitro*, although both of these congeners were much less potent agonists than 17β-estradiol. Concentrations of BDE-47 and BDE-99 that caused proliferation in the E-screen assays were six orders of magnitude greater than concentrations of 17β-estradiol that caused proliferation. Proliferation appeared to be limited by toxicity of BDE-47 and BDE-99 to the MCF-7 cells. It is unknown whether estrogenic effects would be observed before toxic effects *in*

vivo. Meerts et al. (2001) examined the estrogenicity of 17 PBDE congeners using the ER-CALUX assay, and found that eleven congeners exhibited luciferase induction in a dose-dependent manner. PBDEs with the greatest activity were BDE-100, BDE-75, BDE-51, BDE-30 and BDE-119; however, each of these was five orders of magnitude less potent than 17β-estradiol. BDE-47 and BDE-99 were tested, but did not show significant estrogenic activity. Further information on the implications of estrogenic (and also antiestrogenic) activity of PBDEs *in vivo*, will be useful in risk assessments, in establishment of fish consumption advisories, and in future establishment of water quality criteria for the protection of human health.

Toxicity data generated in this study may be useful in establishing ambient water quality criteria or secondary (Tier II) values, should the need for such criteria or values arise. Water quality criteria can be calculated only after a larger database of aquatic toxicity values are available. However, under the Water Quality Guidance for the Great Lakes System Final Rule (U.S. EPA 1995), and under Chapter NR 105, Wisconsin Administrative Code, secondary (or Tier II) acute and chronic values may be calculated as long as a genus mean acute value is available for at least one of three genera in the family Daphnidae, including *Ceriodaphnia* sp. In the present study, BDE-47 was toxic to *C. dubia*, with LC_{50} values of 4.60 and 2.95 µg/L. The reason for the difference between these two acute values is not known. Each of these values are somewhat lower than the 48-hour EC_{50} value (14 µg/L) calculated for *Daphnia magna* exposed to the pentaBDE commercial mixture (in Hardy 2002). *C. dubia* is often more sensitive to organic chemicals than *D. magna*. Also, the pentaBDE commercial mixture contains PBDE congeners other than BDE-47 that may not be as toxic. However, the marine copepod *Acartia tonsa* was shown to be very tolerant to BDE-47, with a 48-hour LC50 value of 2,370 µg/L. In the present study, BDE-47 also affected reproduction of *C. dubia*. The chronic toxicity test yielded a seven-day LOEC value of 2.0 µg/L and an NOEC value of 1.4 µg/L. These values are lower than those published for *Oncorhynchus mykiss* (rainbow trout; LOEC of 16 and NOEC of 8.9 µg/L; in Hardy 2002) and for *D. magna* (LOEC of 9.8 and NOEC of 5.2 µg/L; in Hardy 2002) exposed to the pentaBDE commercial mixture. Information on the effects of individual PBDE congeners on aquatic organisms is still very limited, despite growing evidence that PBDEs are bioaccumulating in aquatic organisms worldwide.

While much is known about concentrations of PBDEs in organisms (e.g., relative concentrations among different congeners, relative concentrations among different species), little is known about where these PBDEs are coming from. This study demonstrated that measurable concentrations of PBDEs can be found in effluents of municipal wastewater treatment plants and industries, and in sediments located near the outfalls where those effluents are discharged to the environment. While concentrations of PBDEs were higher in effluent collected from WWTP B, which receives wastewater from both domestic and industrial sources, than from WWTP A, which receives wastewater from primarily domestic sources, it is possible, given the high volume of PBDEs present in most homes, and the detection of PBDEs in human blood and tissue, that domestic wastewater may act as a significant source of PBDEs to the environment. The presence of PBDEs in industrial effluents likely depends on the nature of the industry and the processes and materials used in manufacturing products. PBDEs were found in effluent collected from Industry C, but not from Industries A or B. Industry A produces cheese and PBDEs would not be expected to be present in such an effluent. Industry B produces plastic products, but no PBDEs were found in this effluent, and it is possible PBDEs are simply not used in any of these

particular plastic products. Industry C produces water filtration products. The source of the PBDEs detected in this effluent are not known.

At the time the present studies were initiated, concentrations of PBDEs in industrial and sewage treatment plant effluents had not been examined. Since then, two studies have been published that examined concentrations of PBDEs in wastewater treatment plant effluents (North et al. 2004 and de Boer et al. 2003). De Boer et al. (2003) examined PBDEs in wastewater treatment plant effluents in the Netherlands, but measured concentrations in particulate matter centrifuged from those effluents. Concentrations of BDE-47 and BDE-209 averaged 22 and 350 µg/kg dry weight, respectively. North et al. (2004) examined concentrations of PBDEs in effluent collected from a wastewater treatment plant in California and found that congeners associated with the pentaBDE commercial mixture (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154) made up 88% of the PBDEs found in the effluent. Those found in highest abundance were BDE-47 and BDE-99, with mean concentrations of 10.5 and 11.2 ng/L, respectively. In the present study, BDE-47 and BDE-99 were the only congeners detected in wastewater treatment plant and industrial effluents (though BDE-209 was not measured) with concentrations ranging from below detection to 4.5 and 3.3 ng/L, respectively. These concentrations are much lower than those shown to cause acute or chronic toxicity to *C. dubia* (LC_{50} of 2.95 µg BDE-47/L and IC_{25} of 2.05 µg BDE-47/L) and much lower than those shown to illicit estrogenic activity (288 µg BDE-47/L and 2,084 µg BDE-99/L).

Because concentrations of PBDEs in effluent may vary temporally, it is important to note that the grab effluent samples collected in this study are representative only of a specific time and place. WWTP A has an average detention time of 24 hours and WWTP B, an average detention time of 16 hours. While these detention times may differ depending on rainfall, amount used for return activated sludge and other factors, they were likely long enough to assume that grab samples were representative of a one-day integrated sample. Concentrations of PBDEs in sediment may vary both temporally and spatially. Collecting sediment from several cm in depth may have integrated some of the temporal variability; however additional samples would need to be collected to address spatial variability.

No PBDEs were detected in sediments surrounding facilities where no PBDEs were detected in effluents (Industries A and B). Where effluent concentrations were highest, they were also highest in nearby sediment; however it was not possible to determine if PBDEs measured in sediment in these areas came from those facilities. Sediment that contained the most PBDE congeners, and the highest concentrations of those PBDE congeners was collected at the site located just upstream ("upstream-near") of Industry C on the lower Sheboygan River. Concentrations of the PBDE congeners measured here (up to 6.0 ng/g) were greater than concentrations of those same congeners measured in sediments of Lake Superior (Song et al. 2004), the Cinca River in Spain (Eljarrat et al. 2004), and seventeen locations in the Netherlands (<0.63 µm fraction only; de Boer et al. 2003), but lower than concentrations of some of the same congeners measured in the River Viskan in Sweden (Sellstrom et al. 1998) and the Guadiana River in Portugal (LaCorte et al. 2003).

Although BDE-209 (the predominant congener in the decaBDE commercial mixture) was not measured in the present study, other researchers have found concentrations of this congener to be

much higher than concentrations of other congeners in sediment (Eljarrat et al. 2004, Song et al. 2004, de Boer et al. 2003, Sellstrom et al. 1998). While the pentaBDE and octaBDE mixtures have been banned in Europe and in the State of California, and are being phased out of production by Great Lakes Chemical Corporation (West Lafayette, IN), there are currently no bans or phase-outs planned for the decaBDE commercial mixture. While it has been generally accepted that the large size of the BDE-209 molecule and its hydrophobicity preclude it from being taken up by organisms, there is growing evidence that BDE-209 is in fact, bioavailable to some organisms. BDE-209 was recently detected in peregrine falcon eggs at concentrations up to 430 ng/g fat (Lindberg et al. 2004).

Recent evidence also suggests that under certain conditions, BDE-209 may debrominate into lower brominated congeners. Soderstrom et al. (2004) showed that BDE-209 is photolytically labile in a variety of matrices including sand, sediment and soil. These authors showed that BDE-209 readily debrominated to form nona- to hexaBDE congeners, including BDE-183 and BDE-154. Photochemical decomposition of BDE-209 has also been demonstrated by Bezares-Cruz et al. (2004), Eriksson et al. (2004), and Hua et al. (2003). Debromination of BDE-209 may also occur within organisms. Debromination of BDE-209 to penta- and hexa-BDE congeners was observed in juvenile carp (*Cyprinus carpio*) following dietary exposure (Stapleton et al. 2004a). Debromination of the penta- and hepta-BDE congeners BDE-99 and BDE-183 to the tetra- and hexa-BDE congeners BDE-47 and BDE-154 has also been observed in juvenile carp (Stapleton et al. 2004b).

The Great Lakes Chemical Corporation has introduced a phosphate-based compound, Firemaster 550, as a replacement for its pentaBDE product and is using a brominated alkylene ether, FF-680, as a replacement for its octaBDE product. A U.S. EPA news release dated November 3, 2003 stated that its preliminary assessment of the Firemaster 550 product indicates it is not persistent, bioaccumulative, or toxic to aquatic organisms. Given the regulatory and scientific history associated with the now ubiquitous PBDE flame retardants, and given recent evidence that PBDE metabolites (e.g., brominated dibenzofurans and methoxylated brominated dibenzofurans; Eriksson et al. 2004) may also be of great concern, it is critical that more detailed testing be conducted to ensure that PBDE-replacement chemicals do not become environmental contaminants of the future.

CONCLUSIONS

This study showed that BDE-47 and BDE-99 can act as estrogen receptor agonists *in vitro* (although both of these congeners were much less potent agonists than 17β-estradiol), that BDE-47 is toxic to *Ceriodaphnia dubia* in the low ppb concentration range, and that effluents and sediments may act as sources of PBDEs to Lake Michigan. While PBDEs measured in effluents were much lower in concentrations than those shown to cause toxicity or estrogenic effects, they may accumulate in sediment, be taken up by benthic organisms and be passed on through the food chain, as demonstrated by the relatively high concentrations measured in Lake Michigan Chinook and Coho salmon (Manchester-Neesvig et al. 2001). Concentrations of PBDEs in the environment and in organisms (including humans) have risen sharply in recent years (Hites 2004). Given the high volume of PBDEs contained in many products currently in use, and the

long life of some of those products, it is possible that concentrations of PBDEs in the environment and in organisms will continue to rise for some time to come, despite bans by some states and a phase-out of production by Great Lakes Chemical of the penta- and octa-BDE commercial mixtures. The potential for continued increases in concentrations of the lower brominated congeners, together with recent evidence for uptake and debromination of BDE-209 (the predominant congener in the deca-BDE commercial mixture, the commercial mixture for which no bans or phase-outs are planned) underline the need for continued study of PBDEs.

RECOMMENDATIONS FOR FUTURE STUDIES

-The tetra- and penta-BDE congeners, BDE-47 and BDE-99 were shown to be weakly estrogenic in the present study, but also toxic to MCF-7 cells at high concentrations. Because recent studies have reported uptake of BDE-209 by some terrestrial organisms, and because BDE-209 has been shown to be less acutely toxic than some of the lower-brominated PBDE congeners, E-screen assays should be conducted with BDE-209 to determine if exposure to this congener may result in estrogenic effects.

-Given reports of endocrine disrupting effects (e.g., thyroid effects) of some PBDE congeners *in vitro*, studies should be conducted to examine implications of such effects *in vivo*.

-Because aquatic toxicity data are limited, toxicity tests should be conducted to examine the effects of PBDEs on a wider variety of aquatic organisms. Such data will be useful in any future calculations of ambient water quality criteria.

-Because the PBDE congener profiles being detected in the environment could not have been predicted based on usage rates of the commercial mixtures, toxicity tests should be conducted with individual PBDE congeners, rather than with commercial mixtures.

-Toxicity tests should initially focus on those PBDE congeners detected most frequently and in the highest concentrations in the environment.

-Studies should be conducted to determine uptake and depuration rates, and sediment bioaccumulation factors (BSAFs) of the commonly detected PBDE congeners in fish. Such data will be useful in any future calculations of human health criteria.

-Glass chambers should be used in toxicity tests conducted with PBDEs.

-Glass chambers should be soaked in acid prior to being washed and used in toxicity tests.

-Flow-through conditions should be used in toxicity testing whenever possible to avoid fluctuations and decreases in concentrations of PBDEs over time.

-Given reports of relatively higher concentrations of BDE-209 in sediments, and of debromination of BDE-209 in the environment and in organisms, tests should be conducted to determine toxicity and fate of this congener in select aquatic (preferably benthic) organisms.

-Given the presence of PBDEs in most homes, the detection of PBDEs in human blood and tissue in other studies, and the finding of PBDEs in municipal WWTP effluents in the present study, additional studies should be conducted that examine concentrations of PBDEs in municipal WWTP effluents. While the number of effluent samples examined in the present study was limited, our findings suggest that over time, municipal WWTPs may act as a significant source of some PBDE congeners to the Great Lakes.

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Table 1. Specific congeners measured for each project objective.

OBJECTIVE	CONGENERS TO BE MEASURED
1. Estrogenic activity	BDE-47, BDE-99
2. Toxicity to *Ceriodaphnia dubia*	BDE-47
3. Concentrations in sediment	BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154
4. Concentrations in effluent	BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154

Table 2. PBDE congeners and their CAS registration numbers.

PBDE Congener	CAS #
BDE-28	41318-75-6
BDE-47	5436-43-1
BDE-66	187084-61-5
BDE-85	182346-21-0
BDE-99	60348-60-9
BDE-100	189084-64-8
BDE-138	182677-30-1
BDE-153	68631-49-2
BDE-154	207122-15-4

Table 3. Mean (±standard deviation) dissolved oxygen, pH and conductivity for each treatment (measured concentrations) measured in the second definitive acute toxicity test that examined the effects of BDE-47 on *Ceriodaphnia dubia*. Dissolved oxygen, pH and conductivity were measured in initial (stock) solutions at 0 and 24 hours, and in final (test chamber) solutions at 24 and 48 hours. Temperature was measured in five chambers located in the center and in each corner of the *C. dubia* board at 24 and 48 hours. Mean temperature was 25.04°C (±0.15, range 24.8 to 25.2, *n*=10).

Parameter	**Treatment (µg BDE-47/L)**								
	0	**0 + DMSO**	**1.16**	**1.95**	**3.37**	**5.42**	**9.35**	**15.4**	**28.7**
Dissolved Oxygen (mg/L)	8.2 (±0.14)	8.2 (±0.12)	8.2 (±0.13)	8.1 (±0.17)	8.1 (±0.21)	8.1 (±0.21)	8.1 (±0.15)	8.1 (±0.26)	8.2 (±0.35)
pH	7.72 (±0.44)	7.82 (±0.35)	7.96 (±0.23)	8.08 (±0.18)	8.16 (±0.14)	8.25 (±0.09)	8.29 (±0.10)	8.27 (±0.12)	8.31 (±0.14)
Conductivity	299 (±17.5)	302 (±16.5)	305 (±22.1)	304 (±18.6)	304 (±14.2)	281 (±35.0)	309 (±13.0)	303 (±13.1)	304 (±10.1)

n=4 for dissolved oxygen and pH measurements
n=3 for conductivity measurements

Table 4. Mean (±standard deviation) dissolved oxygen, pH and conductivity for each treatment (measured concentrations) measured in the third definitive acute toxicity test that examined the effects of BDE-47 on *Ceriodaphnia dubia*. Dissolved oxygen, pH and conductivity were measured in initial (stock) solutions at 0 and 24 hours, and in final (test chamber) solutions at 24 and 48 hours. Temperature was measured in five chambers located in the center and in each corner of the *C. dubia* board at 24 and 48 hours. Mean temperature was 24.96^{o}C (±0.26, range 24.6 to 25.3, *n*=10).

Parameter	**Treatment (µg BDE-47/L)**								
	0	**0 + DMSO**	**1.98**	**2.80**	**4.73**	**7.70**	**13.5**	**19.1**	**34.5**
Dissolved Oxygen (mg/L)	8.2 (±0.20)	8.3 (±0.09)	8.4 (±0.33)	8.5 (±0.00)	8.5 (±0.00)	8.5 (±0.00)	8.6 (±0.07)	8.6 (±0.00)	8.5 (±0.00)
pH	7.95 (±0.48)	8.13 (±0.27)	8.25 (±0.33)	8.11 (±0.04)	8.11 (±0.04)	8.11 (±0.04)	8.11 (±0.04)	8.13 (±0.00)	8.13 (±0.00)
Conductivity	269 (±6.66)	267 (±7.53)	258 (±4.79)	244 (±4.24)	245 (±0.71)	243 (±7.78)	245 (±3.54)	246 (±0.00)	244 (±0.00)

n=4 for 0, 0 (+DMSO), and 1.98 µg/L; *n*=2 for 2.80, 4.73, 7.70, and 13.5 µg/L; *n*=1 for 19.1 and 34.5 µg/L treatments.

Table 5. Mean (±standard deviation) dissolved oxygen, pH and conductivity for each treatment (measured concentrations) measured in the chronic toxicity test that examined the effects of BDE-47 on *Ceriodaphnia dubia*. Dissolved oxygen, pH and conductivity were measured in initial (stock) solutions and in final (test chamber) solutions daily. Temperature was measured in five chambers located in the center and in each corner of the *C. dubia* board daily. Mean temperature was 25.09°C (±0.31, range 24.5 to 25.5, *n*=35).

Parameter	**Treatment (µg BDE-47/L)**						
	0	**0 + DMSO**	**1.4**	**2.0**	**3.3**	**5.3**	**7.9**
Dissolved Oxygen (mg/L)	8.46 (±0.25)	8.62 (±0.36)	8.48 (±0.36)	8.48 (±0.33)	8.56 (±0.30)	8.60 (±0.28)	8.52 (±0.39)
pH	8.32 (±0.34)	8.38 (±0.27)	8.40 (±0.25)	8.42 (±0.24)	8.42 (±0.23)	8.42 (±0.24)	8.49 (±0.25)
Conductivity	283 (±31.6)	297 (±17.7)	297 (±15.3)	302 (±30.2)	296 (±15.2)	291 (±11.6)	315 (±67.9)

n=10 for 0, 0 (+DMSO), 1.4, 2.0, and 3.3 µg/L; *n*=8 for 5.3 and 7.9 µg/L treatments.

Table 6. Location of sample sites. All sample sites were geo-located using a Trimble Pro-XRS global positioning system (GPS) unit. Locational data were collected in the Wisconsin Transverse Mercator (WTM) format with sub-meter accuracy. Data were downloaded from the datalogger using Pathfinder software.

Facility	Location	Latitude North	Longitude West
WWTP A	Facility	43 43 45.916	87 58 12.073
	Upstream	43 43 51.515	87 58 15.628
	Downstream	43 43 41.972	87 58 11.725
WWTP B	Facility	43 43 10.118	87 42 26.777
	Upstream	43 44 41.746	87 42 39.287
	Downstream[a]	-	-
Industry A	Facility	43 44 56.493	87 58 53.844
	Upstream	43 44 47.557	87 58 41.558
	Downstream	43 44 34.432	87 59 03.432
Industry B	Facility	43 43 51.082	87 50 17.470
	Upstream	43 43 51.400	87 50 17.594
	Downstream	43 43 50.127	87 50 13.930
Industry C	Facility	43 44 40.186	87 42 40.731
	upstream – far	43 44 27.381	87 44 39.323
	upstream – near	43 44 41.128	87 42 45.593
	Downstream[b]	43 44 41.746	87 42 39.287

[a]It was not possible to collect a downstream sample for this facility because the effluent pipe is located out in Lake Michigan.
[b]The downstream sample for Industry C is the same as the upstream sample for WWTP B.

Table 7. Date and time of collection of effluent and sediment samples, and associated water chemistry data.

Facility	Location	Date	Time	Type of Water	Temperature (^{o}C)	pH	Conductivity	Total Dissolved Solids	Hardness (mg $CaCO_3$/L)	Dissolved Oxygen (mg/L)
WWTP A	facility	09/24/03	4:40 PM	effluent	18.4	7.87	1400	-	400	9.30
	upstream	09/24/03	4:45 PM	river water	19.1	8.50	703	450	352	9.09
	downstream	09/24/03	4:30 PM	river water	18.9	8.39	877	561	356	8.84
WWTP B	facility	10/09/03	11:45 AM	effluent	18.7	7.15	929	-	248	8.60
	upstream	09/24/03	2:35 PM	river water	15.2	8.15	538	343	268	7.43
	downstream	-	-	-	-	-	-	-	-	-
Industry A	facility	09/24/03	6:00 PM	facility	16.8	8.11	556	-	312	10.00
	upstream	09/24/03	5:30 PM	river water	16.5	8.46	696	445	356	8.77
	downstream	09/24/03	5:40 PM	river water	16.4	8.44	693	444	372	8.25
Industry B	facility	09/24/03	11:20 AM	facility	23.8	8.14	273	193	136	7.76
	upstream	09/24/03	11:30	river	16.1	8.59	669	428	284	11.64

			AM	water						
	downstream	09/24/03	11:40 AM	river water	16.1	8.60	664	424	292	11.07
Industry C	facility	09/24/03	2:50 PM	facility	15.9	7.92	287	112	144	10.15
	upstream – far	09/24/03	1:00 PM	river water	16.9	8.63	660	423	284	12.28
	upstream - near	09/24/03	2:35 PM	river water	16.1	8.30	595	382	284	7.42
	downstream[b]	09/24/03	2:35 PM	river water	15.2	8.15	538	343	268	7.43

[a]It was not possible to collect a downstream sample for this facility because the effluent pipe is located out in Lake Michigan.
[b]The downstream sample for Industry C is the same as the upstream sample for WWTP B.

Table 8. Mean (*n*=2) concentration (in ng/L) and (standard deviation) of individual polybrominated diphenyl ether (PBDE) congeners in municipal and industrial effluents. Where mean concentration was less than the report limit* for a congener, a "<" symbol is presented. One effluent blank sample was collected at each site. Concentrations of individual PBDE congeners were less than report limits in all effluent blanks.

Facility	BDE-28	BDE-47	BDE-66	BDE-85	BDE-99	BDE-100	BDE-138	BDE-153	BDE-154
WWTP A	<	<	<	<	1.2 (0.14)	<	<	<	<
WWTP B	<	4.1 (0.35)	<	<	3.3 (0.42)	<	<	<	<
Industry A	<	<	<	<	<	<	<	<	<
Industry B	<	<	<	<	<	<	<	<	<
Industry C	<	4.5 (2.0)	<	<	2.8 (1.1)	<	<	<	<

*The report limit was 1.0 ng/L for all PBDE congeners except BDE-47. The report limit for BDE-47 was 2.0 ng/L.

Table 9. Mean (*n*=2) concentration (in ng/g) and (standard deviation) of individual polybrominated diphenyl ether (PBDE) congeners in sediment collected upstream or downstream of municipal and industrial facilities. Where mean concentration was less than the report limit[a] for a congener, a "<" symbol is presented. Two sediment blank samples were collected at the downstream location for Industry A on the Mullet River, and at the downstream location for Industry B on the Sheboygan River. Concentrations of individual PBDE congeners were less than the report limits in sediment blank samples collected on the Mullet River. Mean concentrations of BDE-47 and BDE-99 were 0.80 ng/g (±1.1) and 1.7 (±1.5) ng/g, respectively, while concentrations of all other PBDE congeners were less than the report limits in sediment blank samples collected on the Sheboygan River.

Facility	Location	BDE-28	BDE-47	BDE-66	BDE-85	BDE-99	BDE-100	BDE-138	BDE-153	BDE-154
WWTP A	Upstream	<	0.60 (0.01)	<	<	1.1 (0.07)	<	<	<	<
	Downstream	<	0.86 (0.02)	<	<	1.2 (0.07)	<	<	<	<
WWTP B	Upstream	<	3.6 (0.00)	<	<	3.9 (0.14)	1.1 (0.07)	<	0.45 (0.04)	<
	Downstream[b]	-	-	-	-	-	-	-	-	-
Industry A	Upstream	<	<	<	<	<	<	<	<	<
	Downstream	<	<	<	<	<	<	<	<	<
Industry B	Upstream	<	<	<	<	<	<	<	<	<
	Downstream	<	<	<	<	<	<	<	<	<
Industry C	Upstream – far	<	0.97 (0.03)	<	<	1.1 (0.00)	<	<	<	<
	Upstream – near	<	3.8 (0.28)	<	0.58 (0.14)	6.0 (1.3)	1.4 (0.42)	<	0.84 (0.15)	0.61 (0.19)

	Downstream[c]	<	3.6 (0.00)	<	<	3.9 (0.14)	1.1 (0.07)	<	0.45 (0.04)	<

[a]The report limit was 0.40 ng/g for all PBDE congeners.
[b]It was not possible to collect a downstream sample for this facility because the effluent pipe is located out in Lake Michigan.
[c]The downstream sample for Industry C is the same as the upstream sample for WWTP B.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

Figure 10.

Figure 11.

Figure 12.

Figure 13.

Figure 14.

APPENDIX A

GENERAL GLASSWARE WASHING PROCEDURES
AND
ORGANIC CHEMISTRY GLASSWARE WASHING PROCEDURES

GENERAL GLASSWARE WASHING PROCEDURES

Note: The Milli-Q system used to obtain water for blanks does contain a U.V. filter and an activated carbon filter. Analysis of water blanks in the past has not indicated any problem with PBDEs or other organic contaminants in the water.

Acid - Steris CIP 220 - Pump 2
Detergent - Steris CIP 100 - Pump 3

WOHL Inorganic
1. Soak in 1% nitric acid for 1 to 2 hours
2. Wash in cycle 9 and cycle 10.

WOHL Organic and Radiochemistry
1. Wash in cycle 9 and cycle 10.

Biomonitoring
1. Hand wash with tap water, rinse with tap water, rinse in 10% HCl, rinse with tap water , acetone inside, rinse 3 times with RO water

All Other Glass Washing
1. Wash in standard cycle

Cycle 9
8 minutes @ 170°F acid (pump 2)
8 minutes @ 170°F detergent (pump 3)
3 minutes hot tap rinse
3 minutes hot tap rinse

Cycle 10
8 minutes @ 170°F acid (pump 2)
3 minutes cold water rinse
10 sec RO non circulating rinse
10 sec RO non circulating rinse
15 minutes Dry @ 180°F

Standard Cycle
8 minutes @ 170°F acid (pump 2)
8 minutes @ 170°F detergent (pump 3)
3 minutes @ hot tap rinse
3 minutes @ hot tap rinse

RO non circulating rinse
RO non circulating rinse
Dry 15 min @ 180°F

Organic Chemistry
8 minutes @ 170 °F detergent (pump 3)
8 minutes @ 170°F acid (pump 2)
3 minutes @ hot tap rinse
3 minutes @ hot tap rinse
RO non circulating rinse
RO non circulating rinse
Dry 15 minutes @ 180 °F

Extra drying may occur in the drying oven

Acid - Steris CIP 220
Detergent - Steris CIP 100

Pipette Washing
1. Pipette Washer with Alcotab in hot water for 30 minutes
2. Rinse with hot water for 15 minutes
3. Rinse with cold water for 15 minutes
4. Rinse with RO water for 15 minutes

Hand washing refer to hot sink

Note: All glassware is rinsed in hexane or acetone prior to use in sampling/analysis for organic contaminants.

ORGANIC CHEMISTRY GLASSWARE WASHING PROCEDURES
ESS ORG GENOP 0047

Organic Chemistry Department

Revision 2.0

May 1, 2000

Note: The Milli-Q system used to obtain water for blanks does contain a U.V. filter and an activated carbon filter. Analysis of water blanks in the past has not indicated any problem with PBDEs or other organic contaminants in the water.

1.0 Scope and Application

This SOP applies to the washing of all "specialty" glassware in the ESS - Organic Chemistry Department. It includes volumetric pipettes which are placed in a special container for pick-up by the Glassware Department staff.

2.0 Summary

The Department utilizes numerous types of glassware that do not lend themselves to machine washing (e.g., Florisil columns). Such glassware is washed by hand by Department personnel.

3.0 Procedure

3.1 Materials and apparatus

3.1.1 Detergent — ALCONOX, Alconox, Inc., New York, N.Y.

3.1.2 Column Brush

3.1.3 Latex Gloves

3.2 Volumetric Pipettes

3.2.1 Thoroughly rinse, invert and place pipettes in the pipette holder.

3.2.2 Place the holder in an ALCONOX solution and soak for at least eight hours.

3.2.3 The pipette holders will be picked up by Glassware Department staff when necessary.

3.3 Miscellaneous Glassware

3.3.1 Wash with ALCONOX in hot water.

3.3.2 Rinse three times with municipal tap water.

3.3.3 Rinse two times with reverse osmosis water.

3.3.4 If applicable (Florisil and silica gel columns) rinse with solvent (hexane, ethanol, acetone, etc.)

3.3.5 Air dry and store in the proper drawers.

Note: All glassware is rinsed in hexane or acetone prior to use in sampling/analysis for organic contaminants.

Written by: Matthew J. Roach Date: _____

Title: Quality Assurance Officer

Dept: ESS — Organic Chemistry Department

Reviewed by: N/A Date: _____

Title: _____

Dept: _____

Approved by: David P. Degenhardt Date: _____

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APPENDIX B

PBDE TOXICITY TEST CHAMBER GLASSWARE WASHING PROCEDURES

PBDE TOXICITY TEST CHAMBER GLASS WASHING PROCEDURES

Note: Glass beakers used in PBDE toxicity testing are to be washed following the method listed below for the Biomonitoring Unit.

Note: The Milli-Q system used to obtain water for blanks does contain a U.V. filter and an activated carbon filter. Analysis of water blanks in the past has not indicated any problem with PBDEs or other organic contaminants in the water.

Acid - Steris CIP 220 - Pump 2
Detergent - Steris CIP 100 - Pump 3

WOHL Inorganic
3. Soak in 1% nitric acid for 1 to 2 hours
4. Wash in cycle 9 and cycle 10.

WOHL Organic and Radiochemistry
2. Wash in cycle 9 and cycle 10.

Biomonitoring
2. Wash in standard cycle (see below), rinse with RO water, soak in 20% HCl for 24h, rinse with RO water, acetone rinse inside, rinse 3 times with RO water

All Other Glass Washing
2. Wash in standard cycle

Cycle 9
8 minutes @ 170°F acid (pump 2)
8 minutes @ 170°F detergent (pump 3)
3 minutes hot tap rinse
3 minutes hot tap rinse

Cycle 10
8 minutes @ 170°F acid (pump 2)
3 minutes cold water rinse
10 sec RO non circulating rinse
10 sec RO non circulating rinse
15 minutes Dry @ 180°F

Standard Cycle
8 minutes @ 170°F acid (pump 2)
8 minutes @ 170°F detergent (pump 3)
3 minutes @ hot tap rinse

3 minutes @ hot tap rinse
RO non circulating rinse
RO non circulating rinse
Dry 15 min @ 180°F

Organic Chemistry
8 minutes @ 170 °F detergent (pump 3)
8 minutes @ 170°F acid (pump 2)
3 minutes @ hot tap rinse
3 minutes @ hot tap rinse
RO non circulating rinse
RO non circulating rinse
Dry 15 minutes @ 180 °F

Extra drying may occur in the drying oven

Acid - Steris CIP 220
Detergent - Steris CIP 100

Pipette Washing
5. Pipette Washer with Alcotab in hot water for 30 minutes
6. Rinse with hot water for 15 minutes
7. Rinse with cold water for 15 minutes
8. Rinse with RO water for 15 minutes

Hand washing refer to hot sink

Note: All glassware is rinsed in hexane or acetone prior to use in sampling/analysis for organic contaminants.

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APPENDIX C

ANALYSIS OF PBDEs IN TOXICITY TEST SOLUTIONS

SOP: PBDE ANALYSIS OF TOXICITY TEST SOLUTIONS

Wisconsin State Laboratory of Hygiene Organic Chemistry Unit

9/15/04

Determination of PBDE #47 in Test Solutions for Acute and Chronic Toxicity Tests

1. Scope and Application

This method has been verified for the analysis of Polybrominated Diphenyl Ether (PBDE) #47 in 70 to 100 ml test solutions for acute and chronic toxicity tests conducted by the Biomonitoring Unit. It may also be applied to other PBDE congeners and related analytes.

2. Summary of Method

The entire sample, ranging in volume from approximately 25 to 100 mL, is solvent extracted with methylene chloride using a 250 ml separatory funnel. The extract is concentrated under a stream of nitrogen, transferred to iso-octane, and treated with concentrated sulfuric acid. After dilution to an appropriate volume, the extract is analyzed on a gas chromatograph equipped with an electron capture detector.

3. Sample Handling and Preservation

A. Samples are collected in clean, acetone-rinsed 50 or 100 mL amber glass bottles with a Teflon lined cap. Samples are iced or refrigerated at 4 degrees C from time of collection until extraction. Samples should be extracted within 14 days of collection and analyzed within 40 days of extraction.

4. Reagents and Standards

A. Reagents

1. Dichloromethane, hexane, acetone, iso-octane-Pesticide reagent grade.
2. Sodium sulfate: ACS anhydrous granular, 10-60 mesh, stored at 130 C.

B. Standards

1. Standards are purchased as concentrated solutions in sealed ampules from Cambridge Isotope Lab or Wellington Lab.
2. Stock standard solutions are stored in amber bottles at -20 C, and are replaced after one year, or sooner if evaporation or degradation is suspected.

5. Apparatus

A. Separatory funnel - 250 mL with Teflon stopcock.

B. Sample bottle – 50 mL and 100 mL amber glass bottles that are equipped with Teflon lined septum caps.

C. Graduated cylinders, 100 mL, and 50 mL

D. Pipettes - volumetric class A, and Pasteur transfer.

E. Centrifuge Tubes, calibrated, 15 mL capacity

F. Nitrogen blow-down apparatus equipped with heated water bath

G. Drying oven

H. Gas Chromatograph - analytical system complete with temperature programmable GC suitable for use with capillary columns and all required accessories including syringes, columns, gases, electron capture detector and a PC-based integrator.

I. Volumetric flasks

J. Beakers - 100 mL, 150 mL

K. Micro liter syringes – 25 to 250 µL

L. Standard containers (amber bottles - 50 to 200 mL)

6. Quality Control

A. A method blank of organic-free water (polished water or Madison tap water) is analyzed with each batch of 10 samples or less to verify that the background concentration of target compound(s) is below the LOD.

B. A spike of target compound(s) to organic free water is analyzed with each batch of 10 samples or less to verify adequate recovery. Until sufficient data is available to generate statistical limits, limits of 70 to 130% will be used. If recoveries are outside those limits data will be flagged.

C. A surrogate spike of PCB #166 is added to each sample and QC sample prior to extraction to monitor analytical recovery. Surrogates are added to the sample in the separatory funnel prior to extraction. Until sufficient data is available to generate statistical limits, limits of 70 to 130% will be used. If recoveries are outside those limits data will be flagged.

7. Extraction Procedure

A. Rinse all glassware, including separatory funnels, with methylene chloride and discard. Transfer the entire sample to a 250 ml separatory funnel. For expected sample volume of 70 ml or less, add 15 ml methylene chloride to the sample bottle, seal and shake, then add to the sample in the sep funnel. Use 20 mL methylene chloride for sample volumes of 100 mL. Extract the sample by shaking the funnel vigorously for 2 minutes, with periodic venting to release pressure. Allow the organic layer to separate for

a minimum of 10 minutes. Collect the extract in a 250 mL flat bottom flask, or a 150 mL beaker. Repeat the extraction two more times, rinsing the sample bottle each time.

B. Collect the extracted aqueous sample in a 100 mL graduated cylinder, measure the volume and record to the nearest mL.

C. Concentrate the methylene chloride extract to approximately 5 mL under a stream of Nitrogen with addition of iso-octane as keeper, using a water bath if necessary to avoid water condensation into the sample. Transfer to a 15 mL centrifuge tube with iso-octane rinses and continue evaporation to 1 mL. Treat the extract with 1 mL concentrated sulfuric acid.

D. Dilute the extract to an appropriate volume in a volumetric flask and add internal standard to the entire extract if in 5. mL, or to a 1.0 mL aliquot.

8. Analysis – Gas Chromatography

A. GC Conditions for PBDEs.

```
HP 5890-II Gas Chromatograph
60M DB5 column, 0.25 mm ID, 0.1 µm film
Hydrogen carrier gas
Electron Capture Detector; 300 °C
Pressure Programmable Injector; 265 °C
Initial Pressure 40 psi, 1.0 min.  hold
Programmed from 40 psi to 20 psi at
        20 psi/min., then go to constant flow
        mode for remainder of run
Splitless injection; purge on at 0.70 min
Injector volume 1 µL
Oven Temperature Profile:
        Initial Temp 100 °C, hold for 1.0 min
        100 °C to 150 °C at 3 °C/min
        150 °C to 220 °C at 1 °C/min
        220 °C to 280 °C at 5 °C/min, hold for 3
      min
```

B. Calibration

A multi-point internal standard calibration is constructed, using PCB #204 for internal standard and retention time reference peak, and containing a minimum of five calibration levels of the target analyte(s).

APPENDIX D

ANALYSIS OF PBDEs IN EFFLUENTS

ESS ORG METHOD 1608

Determination of Chlorinated Pesticides, Polybrominated Diphenyl Ethers (PBDEs), and PCBs in Wastewater and Storm Run-off Water by Gas Chromatography with an Electron Capture Detector

EPA Method 608 & 608.2 - (July, 1982)

Matrix: Effluents, Wastewater, and Sludge

Scope and Application

1.1. This method may be used to analyze effluents, wastewater and sludge for various chlorinated pesticides, PCBs, and PBDEs.

1.2. This method has been verified for the following compounds but may be applied to other compounds also.

Analyte	LOD (µg/l)	LOQ (µg/l)	PBDE Congener	Report Limit (ng/l)
Aldrin	0.0022	0.0073	# 28	1.0
Gamma BHC (Lindane)	2.0	0.0016	0.0053	# 47
Cis-chlordane	0.0025	0.0083	# 66	1.0
Trans-chlordane	0.0025	0.0083	#100	1.0
p,p'DDD	0.0033	0.011	# 99	1.0
p,p'DDE	0.0030	0.010	# 85	1.0
p,p'DDT	0.0038	0.013	#154	1.0
Dieldrin	0.0030	0.010	#153	1.0
Endrin	0.0045	0.015	#138	1.0
Heptachlor	0.0026	0.0087		
Heptachlor Epoxide	0.0026	0.0087		
Hexachlorobenzene	0.0018	0.0060		
Methoxychlor	0.0060	0.020		
PCBs – Aroclors	0.20	0.67		
Toxaphene	0.20	0.67		

2. Summary of Method:

2.1. A measured volume of sample, approximately one-liter, is solvent extracted with methylene chloride using a separatory funnel. For PBDE analysis, 2 liters are extracted. The extract is concentrated on a rotoevaporator to approximately 5 ml. Two milliliters of iso-octane are added and the volume is reduced under a stream of nitrogen. After Florisil and/or silica gel clean-up the extract is injected on a gas chromatograph equipped with an electron capture detector.

2.2. Regulatory Deviations: The determinative method (EPA 608) calls for hexane as the solvent for the final extract. We use iso-octane. The reasons are two-fold. Iso-octane is recommended as the final solvent by both the AOAC and by the FDA. In addition, the laboratory has found over time that starting an oven temperature program below the boiling point of the solvent yields better chromatographic results.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences

3.2. All laboratory waste, excess reagents and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

4. Sampling Handling and Preservation:

4.1. Samples are collected in 1-liter amber glass bottles with a Teflon lined cap. The cap liners are extracted overnight in methanol prior to sample collection. Samples must be kept refrigerated at 4°C until extraction. All samples must be iced or refrigerated at 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, the sample should be adjusted to a pH range of 5.0 - 9.0 with sodium hydroxide or sulfuric acid. Record the pH and the volume of acid or base used on the sample worksheet.

4.2. If residual chlorine is present, add 80 mg of sodium thiosulfate to the sample.

4.3. Samples should be extracted within 7 days of collection and analyzed within 40 days of extraction.

5. Interferences: **Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that analytes of interest may not be wholly resolved from one another. Positive identifications in unfamiliar samples will be confirmed.**

6. Reagents and Standards:

6.1. Reagents:

6.1.1. Dichloromethane, hexane, acetone, ethyl ether, iso-octane - pesticide grade.

6.1.2. Sodium thiosulfate – anhydrous

6.1.3. Sodium sulfate: ACS anhydrous granular, 10 - 60 mesh, heat at 400°C for four hours and store at 130°C.

6.1.4. Florisil: 60-100 mesh, stored at 130°C.

6.1.5. Silica gel: Fisher Grade 923, 100-200 mesh activated at 130°C.

6.1.6. Glass wool - soxhlet extracted in 50:50 acetone: hexane for 8 hours.

6.1.7. Sulfuric Acid: Certified ACS.

6.2. Standards

6.2.1. Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.2.2. Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in isooctane and dilute to volume in a 10 ml volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.2.3. Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at -15°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.2.4. Stock standard solutions must be replaced after one year, or sooner if comparison with check standards indicates a problem.

7. Apparatus:

7.1. Separatory funnel - 2000 ml with Teflon stopcock and stopper.

7.2. Sample bottle - One-liter amber glass bottles that are equipped with Teflon lined septum caps. The cap liners are extracted overnight in methanol.

7.3. Rotary evaporator equipped with a water bath capable of maintaining 30°C.

7.4. Boiling flask, 500 ml

7.5. Graduated cylinders, 1000 ml, 100 ml, and 50 ml

7.6. Boiling chips - Teflon

7.7. Spatulas

7.8. Pipettes - volumetric class A, and Pasteur transfer.

7.9. Chromatographic columns, 1 cm ID x 40 cm, fitted with a 75 ml reservoir for Florisil and silica gel clean up.

7.10. Centrifuge Tubes, calibrated, 15 ml capacity

7.11. Nitrogen blow-down apparatus equipped with heated water bath

7.12. Drying oven

7.13. Drying columns - glass

7.14. Powder funnel

7.15. Analytical balance - capable of accurately weighing to the nearest 0.0001 g.

7.16. Gas Chromatograph - analytical system complete with temperature programmable GC suitable for use with capillary columns and all required accessories including syringes, columns, gases, electron capture detector and a PC-based integrator.

7.17. Volumetric flasks

7.18. Beakers - 100 ml, 150 ml

7.19. Microliter syringes - 50 µl

7.20. Standard containers (amber bottles - 30 and 60 ml)

8. Quality Control

8.1. For general quality control, procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT".

8.2. The quality assurance procedures followed in this method are a composite of the requirements found in EPA Method 608 - Organochlorine Pesticides and PCBs, the NELAC standards, and the ESS Quality Assurance Manual. The specific quality assurance procedures are outlined below.

8.3. The laboratory operates a formal quality control program. Requirements of this program consist of an initial demonstration of laboratory and analyst capability, detection limit determination and verification, and an ongoing analysis of spiked samples to evaluate and document data quality. The laboratory maintains records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analysis meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a laboratory control sample (i.e., a quality control check standard) must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.4. A method blank, a laboratory control sample (LCS), matrix spike (MS), and a duplicate sample or matrix spike duplicate (MSD) each will be analyzed with each batch of 10 samples or less to evaluate laboratory data quality, and demonstrate that the operation of the measurement system is in control.

8.5. A method blank of organic-free water (Madison tap water) must be analyzed with each batch of samples in order to verify that the background concentration of each target compound is below the LOD. If this criteria is not met, corrective action will be taken to locate and reduce the contamination. The samples associated with this method blank will be re-extracted and reanalyzed or the data will be appropriately flagged.

8.6. For each analytical batch a matrix spike / matrix spike duplicate pair, or a sample duplicate should be analyzed. The decision to prepare and analyze a duplicate samples or an MS/MSD pair must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, the laboratory may use a matrix spike coupled with a sample duplicate. If samples are not expected to contain target analytes, an MS/MSD pair should be analyzed. The precision acceptance criteria will be generated from in-house data. Until enough in-house data is gathered, a limit of 35% RPD will be used. If the results exceed this limit, samples will be re-extracted and reanalyzed or the data will be appropriately flagged.

8.7. The laboratory control sample (Madison tap water), matrix spike and matrix spike duplicate will be spiked with all of the target analytes near the mid-point of the calibration range. A spiking standard separate from those used to generate the calibration curves will be used.

8.7.1. The percent recoveries of the laboratory control sample, matrix spike, and matrix spike duplicate must fall within three standard deviations (the control limit) of the interim limits generated from Table 3 - QC Acceptance Criteria - Method 608, to be considered in control. If they do not fall within the limits, the samples will be re-extracted and reanalyzed or the data will be appropriately flagged. The only exception to this applies if the sample chosen for spiking contains the spiked compound in large amounts (i.e., a sample result equal to or greater than the spiking concentration). Once the laboratory has analyzed 20 to 30 spikes (MS or LCS), in-house limits may be calculated.

8.7.2. A matrix effect is indicated if the LCS recovery data are within the control limits, but the matrix spike data exceed the control limits. Surrogate recoveries from these runs will also be used to help make this determination. If all recoveries for the MS compounds and the surrogate compounds in the LCS are in control, then the batch can be run, with all sample results being flagged due to MS criteria not being met. If LCS recoveries are not all met, a laboratory performance problem is indicated and resolution of the problem must take place before further samples can be analyzed. The samples associated with this LCS will be re-extracted and reanalyzed or the data will be appropriately flagged.

8.8. A surrogate standard is added to each field sample and QC sample prior to extraction in order to monitor analytical recoveries of pesticides. The surrogate is dibutylchlorendate at a concentration of 0.080 µg/ml. The surrogate spike is added to the separatory funnel at the beginning of the analytical procedure. The percent recovery of the surrogate spike must fall within three standard deviations of the in-house generated mean to be in control. If they do not fall within the limits, the samples will be re-extracted and reanalyzed or the data will be appropriately flagged. Until limits are generated for dibutylchlorendate in effluent, accuracy limits of 70 - 130% will be used.

9. Method Calibration

9.1. Aroclor Analysis - Internal Standard

9.1.1. Analysis of PCBs will be based upon a "best fit" Aroclor match. Prepare calibration standards for the appropriate Aroclor or mix of Aroclors at a minimum of four concentrations by adding a volume of the stock standard to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards and dilute to volume with iso-octane. One of the standards should be at a concentration at, or above the MDL. The other concentrations should correspond to the expected range of concentrations found in environmental samples or should define the working range of the detector.

9.1.2. Two four-point curves may be prepared. The low curve with concentrations of 0.100, 0.200, 0.300, and 0.400 µg/ml, and the high curve with concentrations of 0.300, 0.400, 0.800, and 1.50 µg/ml. Internal standards, PCB

congeners #30 and #204 are added to all the standards at concentrations of 0.0142 and 0.0156 µg/ml, respectively.

9.1.3. Inject 1 µL of each calibration standard using the same introduction technique that will be applied to the environmental samples.

9.1.3.1. The calibration points are constructed by calculating an amount ratio and a response ratio for each level in the calibration table.

9.1.3.2. The amount ratio is the amount of the compound divided by the amount of the internal standard at this level.

9.1.3.3. The response ratio is the sum of the areas of the peaks selected for quantification divided by the area of the internal standard at this level.

9.1.3.4. An equation for the curve through the calibration points is calculated using a linear type of curve fit. The results can be used to plot a calibration curve of response ratios versus amount ratios.

9.1.4. A correlation coefficient of 0.995 or greater verifies the acceptability of the curve.

9.1.5. An ongoing verification standard is run after four samples and at the end of the analytical run. If the response of a standard varies by more than $\pm$15% from the expected response, a new calibration curve must be prepared, and all samples analyzed since the last acceptable verification standard must be reanalyzed.

9.1.6. The calibration curve must be verified on each working day by the measurement of one or more calibration standards. If the response for any standard varies from the expected response by more than $\pm$15%, a new calibration curve must be prepared.

9.2. Pesticide Analysis - Internal Standard

9.2.1. Prepare pesticide calibration standards at a minimum of five concentrations for each analyte including the surrogate by adding a volume of the stock standard to a volumetric flask. To each calibration standard, add a known, constant amount of one or more internal standards and dilute to volume with iso-octane. One of the standards should be at a concentration at, or above the MDL. The other concentrations should correspond to the expected range of concentrations found in environmental samples or should define the working range of the detector.

9.2.2. Approximate concentrations of 0.003 - 0.100 µg/ml for all pesticides are employed for the calibration. PCB congener #30 is added to all the standards at a concentration of 0.0142 µg/ml.

9.2.3. Prior to the analysis of calibration standards, a mixture of endrin and p,p-DDT is injected to verify the cleanliness of the system. If the breakdown of either analyte exceeds 20% of the total response for that analyte, corrective action must be taken before analysis can proceed.

$$\% \text{ breakdown of Endrin} = \frac{\text{Resp of Endrin Ketone + Endrin Ald.}}{\text{Resp of Endrin + Endrin Ketone + Endrin Ald.}} * 100$$

$$\%\text{breakdown of p,p'-DDT} = \frac{\text{Resp. of p,p'-DDD + Resp. of p,p'-DDE}}{\text{Resp. of p,p'-DDT + p,p'-DDD + p,p'-DDE}} * 100$$

9.2.4. To ensure that the breakdown of Endrin and p,p'-DDT is less than 20% use a Siltek Deactivated Inlet Liner. The specific liner was a 4 mm Splitless Gooseneck Siltek Deactivated Liner from Restek (product # 20798-214).

9.2.5. Inject 1 µL of each calibration standard using the same introduction technique that will be applied to the environmental samples.

9.2.5.1. The calibration points are constructed by calculating an amount ratio and a response ratio for each level in the calibration table.

9.2.5.2. The amount ratio is the amount of the compound divided by the amount of the internal standard at this level.

9.2.5.3. The response ratio is the height of the peak selected for quantification divided by the height of the internal standard at this level.

9.2.5.4. An equation for the curve through the calibration points is calculated using a linear type of curve fit. The results can be used to plot a calibration curve of response ratios versus amount ratios.

9.2.6. A correlation coefficient of 0.995 or greater verifies the acceptability of the curve.

9.2.7. An ongoing verification standard is run after five samples and at the end of the analytical run. If the response of a standard varies by more than $\pm$15% from the expected response, a new calibration curve must be prepared, and all samples analyzed since the last acceptable verification standard must be reanalyzed.

9.2.8. The calibration curve must be verified on each working day by the measurement of one or more calibration standards. If the response for any standard varies from the expected response by more than $\pm$15%, a new calibration curve must be prepared.

9.3. Toxaphene Analysis - External Standard

9.3.1. Prepare toxaphene calibration standards at a minimum of four concentrations (0.10 - 0.80 µg/ml) by adding a volume of the stock standard to a volumetric flask and diluting to volume with iso-octane. One of the standards should be at a concentration at, or above, the MDL. The other concentrations should correspond to the expected range of concentrations found in environmental samples or should define the working range of the detector.

9.3.2. Inject 1 µl of each calibration standard using the same introduction technique that will be applied to the environmental samples.

9.3.2.1. The calibration points are constructed by plotting the amount (in µg/ml) versus the area sum. The area sum is the total area of the peaks selected for quantification.

9.3.2.2. An equation for the curve through the calibration points is calculated using a linear type of curve fit.

9.3.3. A correlation coefficient of 0.995 or greater verifies the acceptability of the curve.

9.3.4. An ongoing verification standard is run after five samples and at the end of the analytical run. If the response of a standard varies by more than $\pm$15% from the expected response, a new calibration curve must be prepared, and all samples analyzed since the last acceptable verification standard must be reanalyzed.

9.3.5. The calibration curve must be verified on each working day by the measurement of one or more calibration standards. If the response for any standard varies from the expected response by more than $\pm$15%, a new calibration curve must be prepared.

9.4. PBDE Analysis – Internal Standard

9.4.1. Prepare PBDE calibration standards at a minium of five concentrations for each analyte by dilution of stock standard. The lowest standard should be at a concentration at, or just above, the report limit. The other concentrations should correspond to the expected range of concentrations found in samples or should define the working range of the detector: usually from 1.0 to 20 ng/ml. Internal standard PCB congener #204 is added to 1.0 ml aliquots of standard at a concentration of 0.0156 µg/ml just prior to GC analysis.

9.4.2. A linear fit calibration in constructed. A correlation coefficient of 0.980 or greater verifies the acceptability of the curve.

9.4.3. An ongoing verification standard is run every 12 hours and at the end of the analytical run. If, for any analyte detected in samples, the calculated concentration differs from the known concentration by more than 25% a new calibration curve must be prepared, and all samples analyzed since the last acceptable verification standard must be reanalyzed.

9.4.4. The calibration curve must be verified at the beginning of an analytical run by the measurement of one or more calibration standards. If the calculated concentration of a standard differs from its known concentration by more than 25% a new calibration curve must be prepared.

10. Procedure

10.1. Sample Extraction

10.1.1. Rinse all glassware to be used in the extraction with methylene chloride and discard. To the 2 liter separatory funnel add 60 ml of methylene chloride, shake for approximately 30 seconds venting frequently. Discard the methylene chloride.

10.1.2. Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. Pour the entire sample into a 2-liter separatory funnel; add the surrogate spike (dibutyl chlorendate). If analyzing for PBDEs add PCB #166 for the surrogate. For PBDEs, extract two 1-liter bottles and combine the extracts.

10.1.3. Add 100 ml methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Dry the methylene chloride extract by passing it through a drying column fitted with a glass wool plug, half-full of anhydrous sodium sulfate. Collect the extract in a 500 ml boiling flask. Add 60 ml of methylene chloride to the sample bottle, rinse and repeat the extraction procedure a second time. Perform a third extraction in the same manner with 60 ml of methylene chloride. Rinse the sodium sulfate column with 30 ml of methylene chloride.

10.1.4. Determine the original sample volume by refilling the sample bottle to the meniscus mark with water and transfer it to a 1000 ml graduated cylinder and, if necessary, a 100 ml graduated cylinder. Record the sample volume to the nearest 5 ml.

10.1.5. Reduce the methylene chloride extract to about 5 ml on a rotary evaporator. The water bath for the rotary evaporator should be at 30°C. Transfer the extract to a 100 ml beaker, rinse the boiling flask with iso-octane. Add the rinse to the 100 ml beaker. Reduce to 2 ml on a heated water bath under a gentle stream of nitrogen.

10.2. Sample Clean-Up and Fractionation: **Note: If the analysis is for PCBs only, add the concentrated extract from step 10.1.5 above to the silica gel column. It is not necessary to perform the Florisil clean up.

10.2.1. Florisil Clean-Up

10.2.1.1. Fill a 1 cm ID. chromatography column to the base of the reservoir with hexane. Add 1 cm of anhydrous sodium sulfate. Slowly add 8 grams of Florisil to avoid entrapment of air bubbles. Another 1 cm of sodium sulfate is added, on top of the Florisil. Drain the hexane to just above the surface of the top layer of sodium sulfate. Discard the solvent.

10.2.1.2. Add the sample extract and allow it to drain into the adsorbent column. Elute at a rate of 1-2 ml per minute. Rinse the container with 1-2 ml of hexane and add the rinse to the column. When the solvent reaches the top sodium sulfate layer, add 50 ml of 94/6 hexane/ethyl ether. The exact elution volume and make-up may change with each lot of Florisil. The volumes used for a particular batch of Florisil are determined experimentally by spiking standards and analyzing fractions for recovery. Collect the eluate in a 100 ml beaker. Concentrate the first Florisil fraction to approximately 3-5 ml on a heated water bath under a gentle stream of nitrogen. This fraction is now ready for silica gel fractionation. The first Florisil fraction contains PCBs and most of the chlorinated pesticides.

10.2.1.3. After the first elution, solvent reaches the top of the sodium sulfate, change the collection beaker, to a 150 ml beaker, and add a second elution solvent of 100 ml 50/50 hexane/ethyl ether. Elute at 1-2 ml per minute. Concentrate the second Florisil fraction to approximately 3-5 ml on a heated water bath under a gentle stream of nitrogen. Perform a solvent exchange to iso-octane. Transfer the extract to a calibrated centrifuge tube, rinse the beaker with iso-octane and add the rinse to the centrifuge tube. Reduce to a final volume of 2.0 ml under a gentle stream of nitrogen. Add the internal standard. This fraction is now ready for GC-ECD analysis. The second Florisil fraction generally contains dieldrin, endrin, methoxychlor, and dibutyl chlorendate.

10.2.2. Silica Gel Fractionation

10.2.2.1. Weigh an appropriate amount of activated silica gel into a flask. Add enough distilled water to deactivate the silica gel 3.5% by weight and stopper the flask. For PBDEs, add 5.0% by weight of reagent water. The amount of deactivation is determined experimentally and may vary with new batches of silica gel. Occasionally mix gently but thoroughly for at least one hour to equilibrate. Inspect to ensure no clumps are present.

10.2.2.2. Fill a 1 cm ID. column to the base of the reservoir with hexane. Add 1cm anhydrous sodium sulfate, next add 5 g of the deactivated silica gel, and top with an additional 1 cm anhydrous sodium sulfate. Drain the hexane to just above the top sulfate layer. Discard the solvent.

10.2.2.3. Add the extract from the first Florisil fraction and allow it to drain into the adsorbent column. Elute at about 1-2 ml/minute. Rinse the beaker with 1-2 ml of hexane and add the rinse to the column. Collect the eluate in a 100 ml beaker. When the solvent reaches the top sodium sulfate layer, add 50 ml of hexane. Reduce the volume of the eluate to approximately 3-5 ml on a heated water bath, under a gentle stream of nitrogen. Perform a solvent exchange to iso-octane. Transfer the extract to a calibrated centrifuge tube, rinse the beaker with iso-octane and add the rinse to the centrifuge tube. Reduce to a final volume

of 2.0 ml under a gentle stream of nitrogen. Add the internal standard. The first silica gel fraction is now ready for GC-ECD analysis. This fraction generally contains PCBs, p,p'-DDE, hexachlorobenzene, aldrin, heptachlor, and PBDEs. The final extract volume for PBDEs is 1.0 ml.

10.2.2.4. A second elution solvent of 75/25 hexane-ethyl ether is added just as the first eluate reaches the top of the sodium sulfate layer. The exact volume and make-up of the elution solvent is determined as in step 10.2.2.3 above, but is not as critical as the separation has already been completed. Currently, 60 ml is used. The collection beaker should be changed at this point. Elute at about 1-2 ml/minute. Reduce the volume of this fraction to approximately 3-5 ml on a heated water bath, under a gentle stream of nitrogen. Perform a solvent exchange to iso-octane. Transfer the extract to a calibrated centrifuge tube, rinse the beaker with iso-octane and add the rinse to the centrifuge tube. Reduce to a final volume of 2.0 ml under a gentle stream of nitrogen. Add the internal standard. The second silica gel fraction is now ready for GC-ECD analysis. This fraction generally contains cis-chlordane, trans-chlordane, p,p'-DDD, p,p'-DDT, gamma BHC, heptachlor epoxide, and toxaphene.

10.3. Analysis - Gas Chromatography

10.3.1. GC conditions for PCBs, Pesticides and Toxaphene.

10.3.1.1. HP6890 Gas Chromatograph
30m HP-5 column, 0.25mm ID 0.25 µm film thickness
Hydrogen carrier gas
Micro Electron Capture Detector
Injection volume: 1 µL
Inlet: 250 °C
Detector: 300 °C
Temperature program
80°C, hold 1 minute
80-150°C at 10°C/min
150-220°C at 2°C/min
220-275°C at 3°C/min
275°C, hold 1 minute

10.3.1.2. Aroclor identification and quantitation is done by comparing the sample fingerprint to the appropriate Aroclor standard. Quantification is based upon the area sum of selected peaks. Samples with a response ratio greater than the largest calibration standard are appropriately diluted and reanalyzed. Results are reported in µg/l.

10.3.1.3. Pesticide identification of a peak in the sample is $\pm$0.05 minutes of the retention time of the pesticide in the standard. Quantification is based upon area. Samples with a response ratio greater than the largest calibration standard are appropriately diluted and reanalyzed. Results are reported in µg/l.

10.3.1.4. Toxaphene identification is done by comparing the sample fingerprint to the toxaphene standard. Quantification is based upon the area sum of selected peaks. Samples with an area sum greater than the largest calibration standard will be appropriately diluted and reanalyzed. Results are reported in µg/l.

10.3.2. GC Conditions for PBDE Analysis
HP 5890-II Gas Chromatograph
60m DB5 Column, 0.25 mm ID, 0.1 µm film
Hydrogen Carrier Gas
Electron Capture Detector: 300°C
Pressure Programmable Injector: 265°C
Initial Pressure 40 psi, 1.0-minute hold
Programmed from 40 psi to 20 psi at
20 psi/min, then go to constant flow mode for remainder of run.
Splitless injection: purge on at 0.70 min
Injector volume 1 µl
Oven Temperature Profile:

Initial Temperature: 100°C, hold for 1 minute
100°C to 150°C at 3°C/min
150°C to 220°C at 1°C/min
220°C to 280°C at 5°C/min, hold for 3 min

10.3.3. NOTE: Any confirmations that are necessary will be done in conformity with ESS SOP ORG0013 "Confirmation of Non-Mass Spectral Results".

11. Calculations:

11.1. The equation used to calculate the amount of PCB is:

Aroclor conc.(µg/L) = ((Resp. Ratio - b)/m)*(Amt I.S. (µg)*(Multiplier))

Where: Resp. Ratio = (Area sum of peaks/Area of I.S.)
I.S. = Internal Standard
b = y-intercept of the linear regression of amount ratios vs. response ratios
m = Slope of the linear regression of response ratios vs. amount ratios
Multiplier = (Final volume (ml)) / (Volume extracted (L))

11.2. The equation used to calculate the amount of pesticides is:

Pesticide conc. (µg/L) = ((Resp. Ratio - b)/m)*(Amt I.S. (µg)*(Multiplier))

Where: Resp. Ratio = (Area of peaks/Area of I.S.)
I.S. = Internal Standard
b = y-intercept of the linear regression of amount ratios vs. response ratios
m = Slope of the linear regression of response ratios vs. amount ratios
Multiplier = (Final volume (ml)) / (Volume extracted (L))

11.3. The equation used to calculate the amount of toxaphene is:

Toxaphene conc. (µg/L) = (Resp. - b)/m)*(Multiplier)

Where: Resp. = Area sum of peaks
b = y-intercept of the linear regression of amount vs. response
m = Slope of the linear regression of response vs. amount
Multiplier = (Final volume (ml)) / (Volume extracted (L))

11.4. The equation used to calculate the amount of PBDE is:

PBDE conc. (ng/l) = (Resp. Ratio – b)/m*(Amt I.S. (ng))*(Multiplier)

Where: Resp. Ratio = (Area of peak/Area of I.S.)

I.S. = Internal Standard

b = y-intercept of the linear regression of response ratio vs. amount ratio

m = Slope of the linear regression of response ratio vs. amount ratio

Multiplier = Final volume (ml)/Volume extracted (l)

12. **Data Management:** Data is collected using a PC-based Chemstation integrator. It is then transferred to the laboratory worksheet. All data is reviewed (by peers or section supervisors) and then manually entered onto the Laboratory's LIMS system.

13. **Definitions:** General definitions of other terms that may be used in this method are found in Section 19 of the SLH Quality Assurance Manual.

14. **Method Performance:** Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure "ESS ORG QA0012 LOD and LOQ Determinations". Data generated within the last two years will be located in the filing cabinet in the Department supervisor's cubicle. Any data older than two years is stored in the Department filing cabinet in the basement.

15. **References:**

15.1. "Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater - Method 608", US EPA EPA/600/4-82/057, July 1982.

15.2. "Methods for the Determination of Nonconventional Pesticides in Municipal and Industrial Wastewater - Vol. I - Method 608.2"

15.3. "Separatory Funnel Liquid-Liquid Extraction", EPA Method 3510C (Revision 3, December, 1996).

15.4. "Florisil Cleanup", EPA Method 3620B, (Revision 2, December, 1996).

15.5. "Silica Gel Cleanup", EPA Method 3630C, (Revision 2, December, 1996).

15.6. "Quality Assurance Procedures and Policies", The ESS QA Manual.

15.7. "Constitution, Bylaws, and Standards", National Environmental Laboratory Accreditation Conference, (July 1999)

16. **Tables, figures, diagrams, charts, checklists, appendices:** Not Applicable**.**

17. Signatory Page:

17.1. Written by: Carol Buelow Date: 10/1/03
Title: Chemist-Advanced
Unit: ESS Organic Chemistry

17.2. Reviewed by: Carol Buelow Date: 9/27/04
Title: Chemist-Advanced
Unit: ESS Organic Chemistry

17.3. Approved by: Steve Geis Date: 10/7/04
Title: ESS Organic Supervisor
Unit: ESS Organic Chemistry

APPENDIX E

ANALYSIS OF PBDEs IN SEDIMENTS

ESS ORG METHOD 1510

Sediment and Soil for Pesticide, PCB and PBDE Residues

Matrix: Sediment and Soil

1. Scope and Application

17.4. This method may be used to analyze soil and sediment for various chlorinated pesticides, PCBs and polybrominated diphenyl ethers (PBDEs). Analysis for PCBs includes: Aroclor mixtures by megabore column (section 1.3, below), individual congeners contained in the "Mullin" mix by capillary/ECD (section 1.4), and selected co-planar (or toxic) congeners by multi-dimensional GC/ECD (section 1.5). PBDE congeners are listed in section 1.6.

17.5. Pesticides by Capillary Column Chromatography

Compound	LOD (µg/g)	LOQ (µg/g)	Report Limit (µg/g)
dieldrin*	0.012	0.041	
o,p-DDE			0.01
p,p-DDE	0.0050	0.016	
o,p-DDD			0.01
p,p-DDD	0.010	0.033	
o,p-DDT			0.01
p,p-DDT	0.014	0.045	
cis-chlordane	0.0085	0.028	
trans-chlordane	0.0085	0.028	
cis-nonachlor	0.0085	0.028	
trans-nonachlor	0.0085	0.028	
aldrin			0.01
endrin	0.014	0.047	
heptachlor			0.01
heptachlor epoxide			0.01
hexachlorobenzene		0.0085	0.028
alpha-BHC	0.0085	0.028	
gamma-BHC	0.011	0.037	
methoxychlor	0.050	0.16	
toxaphene			1.0
endosulfan I			0.01
endosulfan II			0.01
endosulfan sulfate			0.01

*dieldrin is analyzed on a packed column

17.6. PCBs by Megabore Column Chromatography

Aroclor	LOD(µg/g)	LOQ(µg/g)

1016	0.024	0.080
1232	0.024	0.080
1242	0.024	0.080
1248	0.024	0.080
1254	0.024	0.080
1260	0.024	0.080
1268	0.024	0.080

17.7. PCB Congeners by Capillary Column Chromatography

17.7.1. Sediment

BZ # (Ref 15.5)	LOD(ng/g)	LOQ(ng/g)	BZ # (Ref 15.5)	LOD(ng/g)	LOQ(ng/g)
#3	6.0	19.0	#135/144	0.20	0.64
#4/10	0.90	2.9	#123/149	0.20	0.64
#7/9	0.20	0.64	#118	0.30	0.96
#6	0.30	0.96	#146	0.30	0.96
#8/5	0.60	1.9	#132/153/105	0.20	0.64
#19	0.20	0.64	#141	0.15	0.48
#18	0.20	0.64	#137/176	0.20	0.64
#15/17	0.35	1.1	#163/138	0.30	0.96
#24/27	0.30	0.96	#158	0.30	0.96
#16/32	0.70	2.2	#178	0.20	0.64
#26	0.30	0.96	#187/182	0.20	0.64
#25	0.40	1.3	#183	0.20	0.64
#28/31	0.40	1.3	#128	0.20	0.64
#33	0.40	1.3	#167	0.30	0.96
#53	0.30	0.96	#185	0.20	0.64
#51	0.20	0.64	#174	0.20	0.64
#22	0.30	0.96	#177	0.20	0.64
#45	0.20	0.64	#202/171	0.20	0.64
#46	0.20	0.64	#172	0.20	0.64
#52	0.20	0.64	#180	0.20	0.64
#49	0.15	0.48	#193	0.20	0.64
#47/48	0.30	0.96	#199	0.20	0.64
#44	0.20	0.64	#170/190	0.20	0.64
#37/42	0.30	0.96	#198	0.15	0.48
#41/71/64	0.30	0.96	#201	0.20	0.64
#40	0.20	0.64	#203/196	0.25	0.80
#63	0.20	0.64	#208/195	0.15	0.48
#74	0.20	0.64	#207	0.20	0.64
#70/76	0.20	0.64	#194	0.15	0.48
#66	0.30	0.96	#206	0.15	0.48

#95	0.20	0.64
#91	0.20	0.64
#56/60	0.30	0.96
#92/84	0.40	1.3
#89	0.30	0.96
#101	0.25	0.80
#99	0.20	0.64
#83	0.20	0.64
#97	0.20	0.64
#87	0.20	0.64
#85	0.20	0.64
#136	0.45	1.4
#77/110	0.30	0.96
#82	0.20	0.64
#151	0.20	0.64

17.7.2. Soil

BZ # (Ref 15.5)	LOD(ng/g)	LOQ(ng/g)	BZ # (Ref 15.5)	LOD(ng/g)	LOQ(ng/g)
#3	3.0	9.6	#123/149	0.10	0.32
#4/10	0.50	1.6	#118	0.20	0.64
#7/9	0.15	0.48	#146	0.10	0.32
#6	0.20	0.64	#132/153/105	0.20	0.64
#8/5	0.40	1.3	#141	0.060	0.19
#19	0.15	0.48	#137/176	0.10	0.32
#18	0.15	0.48	#163/138	0.25	0.80
#15/17	0.20	0.64	#158	0.15	0.48
#24/27	0.10	0.32	#178	0.10	0.32
#16/32	0.70	2.2	#187/182	0.080	0.26
#26	0.15	0.48	#183	0.080	0.26
#25	0.15	0.48	#128	0.080	0.26
#28/31	0.25	0.80	#167	0.10	0.32
#33	0.15	0.48	#185	0.080	0.26
#53	0.15	0.48	#174	0.070	0.22
#51	0.10	0.32	#177	0.080	0.26
#22	0.25	0.80	#202/171	0.070	0.22
#45	0.10	0.32	#172	0.10	0.32
#46	0.10	0.32	#180	0.15	0.48
#52	0.15	0.48	#193	0.10	0.32
#49	0.10	0.32	#199	0.080	0.26
#47/48	0.10	0.32	#170/190	0.080	0.26
#44	0.10	0.32	#198	0.15	0.48
#37/42	0.10	0.32	#201	0.10	0.32
#41/71/64	0.15	0.48	#203/196	0.15	0.48
#40	0.10	0.32	#208/195	0.070	0.22
#63	0.10	0.32	#207	0.060	0.19
#74	0.10	0.32	#194	0.060	0.19
#70/76	0.10	0.32	#206	0.060	0.19
#66	0.20	0.64			
#95	0.10	0.32			
#91	0.15	0.48			
#56/60	0.10	0.32			
#92/84	0.15	0.48			
#89	0.10	0.32			
#101	0.20	0.64			
#99	0.10	0.32			
#83	0.10	0.32			

#97	0.10	0.32
#87	0.10	0.32
#85	0.10	0.32
#136	0.45	1.4
#77/110	0.20	0.64
#82	0.10	0.32
#151	0.10	0.32
#135/144	0.10	0.32

17.8. "Toxic" PCB Congeners by Multidimensional Gas Chromatography: The toxic congener list includes the twelve congeners in the World Health Organization (WHO) 1998 list, plus congener #180. There is overlap with the list in Section 1.4, which also includes congener #118, #167, and #180.

BZ # (e)	LOD(ng/g)	LOQ(ng/g)
#77	0.20	0.67
#81	0.20	0.67
#105	0.25	0.83
#114	0.20	0.67
#118	0.35	1.2
#123	0.35	1.2
#126	0.15	0.50
#156	0.25	0.83
#157	0.15	0.50
#167	0.20	0.67
#169	0.15	0.50
#180	0.30	1.0
#189	0.20	0.67

17.9. PBDE Congeners by Capillary Column Chromatography

Compound	Report Limit (ng/g)
PBDE #28	0.40
PBDE #47	0.40
PBDE #66	0.40
PBDE #100	0.40
PBDE #99	0.40
PBDE #85	0.40
PBDE #154	0.40
PBDE #153	0.40
PBDE #138	0.40

18. Summary of Method:

18.1. Soil or sediment samples are air dried and homogenized by sieving. The sample is then soxhlet extracted with hexane/acetone for sixteen hours. After concentrating with a roto-evaporator the extract is run through a column containing Florisil. If pesticides are of interest, a second Florisil fraction will be collected. The first fraction is concentrated and run through a column containing silica-gel. The final extract or extracts are concentrated and injected onto a gas chromatograph equipped with an electron-capture detector. The type of column and GC used varies depending on particular analytes.

18.2. Regulatory Deviations: This section is not applicable to this method.

19. Safety and Waste Management:

19.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences.

19.2. All laboratory waste, excess reagents and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

20. Sampling Handling and Preservation: **Submit in glass wide mouth jars. A quart Mason jar with a Teflon liner is preferred although one with an aluminum foil liner is acceptable. Sample are kept on ic or refrigerated at 4^{o}C from the time of collection until air-drying for analysis.**

21. Interferences:

21.1. Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that analytes of interest may not be wholly resolved from one another. In unfamiliar samples positive identifications will be confirmed.

21.2. High levels of sulfur can also cause contamination in some samples. A small amount of copper shot added to the soxhlet can mitigate this problem.

21.3. **CAUTION:** If analyzing for pesticides copper shot should be avoided. Copper can be destructive to some pesticides.

22. Reagents and Standards:

22.1. Hexane, acetone, ethyl ether, isooctane - pesticide grade.

22.2. Sodium sulfate: ACS granular, stored at 130°C .

22.3. Florisil: PR grade 60-100 mesh, stored at 130°C .

22.4. Silica gel: Davison Grade 923, 100-200 mesh activated at 130°C .

22.5. Stock Standard Solutions:

22.5.1. Prepare stock standard solutions by accurately weighing about 10 mg of pure material. Dissolve the material in isooctane and dilute to volume in a 100 mL volumetric flask.

22.5.2. Transfer the stock standard solution into a Teflon-sealed screw cap amber bottle. Store stock standards in a freezer.

22.5.3. Stock standard solution must be replaced every year or when signs of degradation or evaporation appear.

23. Apparatus:

23.1. Mortar and pestle

23.2. Number 10 sieve

23.3. 500 mL boiling flasks

23.4. Soxhlet condenser and thimbles

23.5. Florisil and silica gel columns

23.6. Calibrated 15 mL centrifuge tubes

23.7. Micro-syringes

23.8. Nitrogen blow-down apparatus

23.9. Volumetric flasks - 10, 25, 100 mL

23.10. Megabore column GC/ECD - HP5890 equipped with a PC-based Chemstation integrator

23.11. Capillary column GC/ECD - HP5890 equipped with a PC-based Chemstation integrator or equivalent.

23.12. Siemens Multi-Dimensional GC/ECD equipped with a Spectra-Physics dual channel integrator.

24. Quality Control

24.1. For general quality control procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT".

24.2. Matrix spikes:

24.2.1. 2-10 mL of a solution containing spiked parameters (acetone solvent) is added to the dried sample after it has been weighed and placed in the soxhlet extraction thimble. Allow the solvent to evaporate before extraction. The spike solution for PCB congeners consists of a mixture of Aroclors 1232, 1248, and 1262 at concentrations of 0.250, 0.180, and 0.180 mg/l, respectively, or higher but at the same ratios. Matrix spikes of toxic congeners must be done on a separate aliquot of sample.

24.2.2. The spike solution for Aroclor PCB analysis should contain the Aroclor that, in the opinion of the analyst, is most likely to be present in the sample.

24.3. For PCB congener analysis, surrogate standards are added to all samples to monitor analytical recoveries. The surrogate spike solution consists of PCB congeners #14, #65, and #166 at nominal concentrations of 100, 25, and 25 ng/mL, respectively. From 1 to 5 mL of this solution is added to every sample as described for matrix spikes.

24.4. For each batch of approximately 10 samples a duplicate sample is analyzed.

24.5. If any of the parameters in section 8.1, 8.2, 8.3 and 8.4, exceed their limits (See ESS Quality Assurance Manual.) the samples will be re-analyzed or appropriately flagged.

24.6. For each batch of approximately 10 samples a method blank will be analyzed. If any analytes of interest are found above their LOD (or report limit) the samples will be re-analyzed or the data appropriately flagged.

24.7. All data pertinent to preparation of standards is recorded in the "Preparation of Standards" log book. Pertinent data is to include date of preparation, origin of parent

solution/primary standard, aliquot and dilution information, all weighings and tares, and purity.

24.8. When fresh stock solutions are prepared from primary standards a log sheet is begun noting the origin, purity, date of preparation, and pertinent weighings and dilutions. Subsequent intermediate and working (or spiking) solutions prepared from the stock will also be documented on this same log sheet. In the same manner discarded solution's listings are marked with a note mentioning reason and date of discard. Log sheets representing discarded parent (stock) solutions are removed from the active portion of the "Preparation of Standards" and filed by name.

24.9. Before introducing freshly prepared standards to routine analysis, they should be compared to current working standards where applicable and checked for impurities (unwanted chromatographic responses). Record response, retention, and all pertinent data in the Q.C. log book and file chromatograms appropriately.

24.10. For Aroclor PCB analysis the method will be monitored by adding a surrogate. Tetrachloro-m-xylene will be spiked into each sample and calculated for recovery. Statistical limits are kept and the system will be investigated if the surrogate recoveries fall outside those limits.

25. Method Calibration

25.1. Aroclor Analysis

25.1.1. Aroclor analysis is done on the megabore column using single point quantitation. The response of selected peaks in the sample chromatogram is directly compared to the same peaks in the standard. The response of the sample must be within 20% of the response of the standard for valid calculations. Mixtures of Aroclors may be required to achieve the best match of GC fingerprints of sample and standard.

25.1.2. Prior to analyzing any samples the linearity of the chromatographic system must be demonstrated. Initial demonstration of linearity involves injecting a series of five standards each consisting of equal concentrations of Aroclors 1016 and 1260. The concentrations of these standards should span the anticipated analytical range. To evaluate the linearity, calculate the relative standard deviation (%RSD) of the response factors (RF) for the five standards:

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Where:

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and

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25.1.3. If the %RSD is less than or equal to 20% over the analytical range, then linearity through the origin may be assumed and the analyses may proceed.

25.1.4. After an initial demonstration of linearity, subsequent analyses may begin only after a verification of the linearity is performed. This is done by determining the response factor for a verification standard (one of the Aroclor 1016 + 1260 mixes) with a concentration at the midpoint of the analytical range. If the RF of the verification standard is within $^{+}$15% of the average RF determined above, then the analyses may proceed.

25.2. Pesticide analysis - Internal Standard: Pesticides are analyzed on a capillary GC using a calibration curve of at least five points and employing a linear fit. A correlation coefficient of 0.990 or greater is required before analysis can begin. PCB congeners #30 and #204 at nominal concentrations of 14 and 16 ng/mL respectively are used as retention time reference peaks and as internal standards for quantitation. Pesticides eluting prior to and including p,p'-DDE use congener #30 as the internal standard, those eluting after p,p'-DDE use #204. Ongoing calibration checks should quantitate to within $^{+}$15% of the initial calibration value for each analyte of interest.

25.3. PCB Congener analysis - Internal Standard

25.3.1. The single point PCB calibration standard consists of a dilution of a stock solution of Aroclors 1232, 1248, and 1262 at 183 µg/mL which was supplied by M. Mullin in June, 1994. See **Table II** for congener composition of the stock solution. The diluted standard contains Aroclors 1232, 1248, and 1262 at 0.225, 0.162, and 0.162 µg/ml for a total of 0.549 µg/mL PCB.

25.3.2. Quantitation of congeners#128 and #167 requires the addition of individual standards of these congeners to the calibration mix, at nominal concentrations of 4 ng/mL and 2 ng/mL, respectively. The total concentration of these congeners in the calibration mix must also include the contribution from the Aroclors. This contribution is 0.30 ng/mL of #128 and 0.15 ng/mL of #167. This standard also contains PCB congener #30 at a nominal concentration of 0.012 mg/l (12 ng/mL), and PCB congener #204 at 0.013 mg/l (13 ng/mL) which are used as retention time reference peaks and as internal standards for quantitation. Congeners eluting prior to and including #77/110 use congener #30 as internal standard, those eluting after #77/110 use congener #204 as internal standard. The calibration table contains the concentration in ng/mL of each congener in the mix, including internal standards, as well as surrogates #14, #65, and #166 at nominal concentrations of 32, 7, and 8 ng/mL. See **Table I**

25.4. Toxic congener analysis - Internal Standard: Thirteen "toxic" or co-planar PCB congeners listed in Section 1.5 are analyzed on a multi-dimensional GC equipped with dual columns in series. The PCB congeners are quantitated on the second column using a mix of pure standards, single point calibration, and PCB #30 as internal standard. PCB #204 is used as a retention time reference peak. GC Method 1 with cuts for all the toxic congeners is calibrated at 10 ng/mL. GC Method 2 with cuts for #81, #77, #114, #126, #167, #157/204, #169, and #189 is calibrated at 5.0 ng/mL. Linearity is verified by running standards at concentrations from 1.0 to 15 ng/mL. Sample concentrations at or near the

LOD will be calculated using the 1.0 ng/mL standard. If the response of a sample analyte exceeds the linear range of the instrument the extract will be diluted to bring it into range.

25.5. PBDE Congeners – Internal Standard: A multi-point internal standard calibration (minimum of three points) using PCB #204 as internal standard and RT reference and using a linear fit, is used for PBDE analysis. On going calibration checks should quantitate to within ±25% of the calibration value for each analyte.

26. Procedure

26.1. Sample Extraction

26.1.1. Place the sample in a shallow pan to a depth of about one inch and allow to air dry. Drying time is usually two to five days depending upon the moisture content. Do not take the sample to complete dryness! The sample should be dry enough to easily pass through a #10 sieve (usually 10-30% moisture). However, sandy soils may be less than 10% moisture.

26.1.2. After drying, the sample is passed through a #10 sieve. Any remaining material which does not pass through the sieve is discarded.

26.1.3. A 10 to 25 g portion of the homogenized sample is taken and analyzed for moisture content. This is done by weighing 10 to 25 gm of soil into a weighed 100 ml beaker and placing it into a 103 °C oven for at least 10 hours. The dried soil and beaker is then weighed. Percent moisture is calculated by the following formula:

(wet weight - dry weight) x 100 / wet weight

If the moisture is greater than 30% the sample is re-dried and a moisture determination is again made. If the moisture content is less than or equal to 30%, a 40-50 g portion is weighed into an acetone washed paper soxhlet extraction thimble.

26.1.4. The sample filled thimble is next placed in an acetone washed soxhlet extraction apparatus. A few glass beads or boiling chips are placed in the soxhlet flask with 300ml 50/50 v/v acetone and hexanes mixture. Granular activated copper may also be added to minimize sulfur interferences.

26.1.5. The apparatus is placed on a hot plate and the sample is extracted for at least 16 hours. The temperature is adjusted so that the soxhlet cycles 5-8 times an hour.

26.1.6. Following the extraction, the sample (now in the acetone-hexanes solvent mixture) is concentrated under a gentle stream of nitrogen or in a rotary evaporation apparatus. At this point it is important to remove as much acetone and water as possible. After concentrating to ca. 10 ml, 10 ml iso-octane may be added and the sample re-concentrated to aid in the removal of acetone. Anhydrous sodium sulfate is added to aid in the removal of water.

26.2. Sample Cleanup and Fractionation

26.2.1. Florisil Cleanup

26.2.1.1. Fill a dry 20 mm I.D. chromatography column to the base of the reservoir with hexanes. Add about 1.2 cm of anhydrous sodium sulfate. Slowly add 22 g of Florisil (about 45-55 ml) avoiding entrainment of air bubbles. Add 2.5 cm sodium sulfate to the top and draw the hexanes to just above the surface of the top layer of sodium sulfate.

26.2.1.2. Add the sample extract (usually 5-10 ml in volume). Open the stopcock and elute at about 5 ml/minute. Rinse the sample container twice with a small amount of hexanes and add the rinses to the column. When the solvent reaches the sulfate layer, add 200 ml of 94/6% hexane-ethyl ether elution mixture. Collect the eluate in a 250 ml beaker. This first Florisil fraction contains PCBs and most of the chlorinated pesticides. The rest of the chlorinated pesticides are in the second elution as noted in step 10.2.1.3 below. Exact elution volume and makeup may change upon arrival of new Florisil lots. The volume used for a particular batch of Florisil is determined experimentally by spiking standards and analyzing fractions for recoveries.

26.2.1.3. If dieldrin, endrin, or methoxychlor is to be analyzed, elute a second fraction using 150 ml of 50% ether/hexane. If endosulfan or endosulfan sulfate is to be analyzed, 150 ml of 100% ethyl ether should be used to elute the column. Add the second elution solvent when the first has reached the top sulfate layer. Change receiving beakers directly following the addition.

26.2.1.4. If the samples are being analyzed for PCB-Aroclor identification only, the first Florisil fraction may be analyzed by GC-ECD. If the clean up has not been adequate this fraction should be taken through the silica gel clean up. If the samples are to be analyzed for PCB congeners, or pesticides and PCB Aroclor identification, a silica gel clean up must be done. The second Florisil fraction is also ready for analysis - see Section 10.3.3, Gas Chromatography.

26.2.2. Silica Gel Fractionation

26.2.2.1. Silica Gel activation is accomplished by heating at 130 °C for 12 hours. Activated gel is maintained at 130 °C until used.

26.2.2.2. Weigh an appropriate amount of activated silica gel into a stoppered flask. The amount of deactivation will change depending on the target analytes, and also may vary with new batches of gel. New batches of silica gel are calibrated to determine exact deactivation levels. Currently, if PBDEs are not requested, deactivate with 3.5% organic-free water. If PBDEs are to be analyzed, deactivate with 5.0% organic-free water. Mix gently but thoroughly, allowing one hour to equilibrate. Inspect to be sure no clumps are present.

26.2.2.3. Fill a 10 mm I.D. column to the base of the reservoir with the hexanes. Add 1 cm anhydrous sodium sulfate, 5 g of the deactivated silica gel, and then 1 cm anhydrous sodium sulfate. Drain the mixed hexanes to just above the top sulfate layer.

26.2.2.4. Add the first Florisil fraction. Elute at about 1-2 ml/minute. When the initial sample extract is just above the top sulfate layer, add 50 ml hexane and continue eluting at about 1-2 ml/minute. At 3.5% water, this eluate (SG1) will contain PCBs and pp'-DDE. At 5.0% water, the

SG1 will contain PCBs, PBDEs, p,p-DDE, p,p-DDT and some of the ? and a chlordane.

26.2.2.5. A second elution of 75/25% hexane-ethyl ether mix is added just as the first eluate reaches the top of the sulfate layer. The exact volume is determined as in step 10.2.2.4 above, but is not as critical as the separation has already been completed. Currently 60 ml is used. The receiving beaker should be changed at this point. This second fraction (SG2) contains chlordanes and DDT plus its metabolites.

26.2.2.6. Reduce the volume of each fraction under a gentle stream of nitrogen and transfer to the appropriate volumetric glassware for GC-ECD analysis. For samples extracted for toxaphene, only the SG2 fraction need be analyzed.

26.3. Gas Chromatography for Megabore Column PCB and Pesticide Analysis

26.3.1. Megabore Column GC Conditions:

HP6890 GC with Electron Capture Detector
Column: DB-5 15m x 0.45m ID, 1.27 µm thickness
Carrier gas: Hydrogen
Detector make-up gas: Nitrogen
Splitless injector, purge on at 0.75 min
Constant flow @ 4.8 mL/min
Typical Temperature Settings:
Oven Temp: Initial temp 85°C, hold for 1 min
85°C to 165°C at 16°C/min
165°C to 275°C at 4°C/min, hold for 2.0 min
Injector: 265°C
Detector: 300°C

26.3.2. The second silica gel fraction contains chlordanes, nonachlors, some DDT metabolites, methoxychlor and toxaphene. A capillary column is necessary to separate the individual pesticides. See Section 10.4.

26.3.3. Analyze the second Florisil fraction for dieldrin and endrin using the GC conditions described in Section 10.4.

26.3.4. Results are usually calculated on a dry weight basis. The following equation is used in determining the results of a given PCB or pesticide.

Conc. = (R.sam/R.std)(V.sam/I.sam)(I.std * C.std/W.sam) / % solids

where:

R.sam = sample response
V.sam = sample extract vol. (ml)
R.std = std response
I.sam = sample inj. vol. (µL)
I.std = std inj. vol. (µL)
C.std = std conc (mg/L)
W.sam = wet wght of sample (gm)

% solids = (100 - %moist.)/100

The final results are expressed in µg /gm (parts per million).

26.4. Gas Chromatography for Pesticide Analysis by Capillary Column

26.4.1. GC Conditions

HP 5890-II Gas Chromatograph
60M DB1 column, 0.25 mm ID, 0.1 µm film
Hydrogen carrier gas
Electron Capture Detector; 300°C
Pressure Programmable Injector; 265°C
Initial Pressure 40 psi, 1.0 min. hold
Programmed from 40 psi to 20 psi at 20 psi/min., then go to constant flow mode for remainder of run
Splitless injection; purge on at 0.70 min
Injector volume 1 µL
Oven Temperature Profile:
Initial Temp 100°C, hold for 1.0 min
100°C to 150°C at 3°C/min
150°C to 220°C at 1°C/min
220°C to 280°C at 5°C/min, hold for 3 min

26.4.2. Calculations, see Section 10.5.5.

26.4.3. Confirmation of correct pesticide identifications may be done on a 60 m DB-5 column using GC conditions as given in Section 10.5.1.

26.5. Gas Chromatography for PCB and PBDE Congener Analysis by Capillary Column

26.5.1. GC Conditions

HP 5890-II Gas Chromatograph
60M DB5 column, 0.2 mm ID, 0.1 µm film
Hydrogen carrier gas
Electron Capture Detector; 300°C
Pressure Programmable Injector; 265°C
Initial Pressure 40 psi, 1.0 min. Hold
Programmed from 40 psi to 20 psi at 20 psi/min., then go to constant flow mode for remainder of run
Splitless injection; purge on at 0.70 min
Injector volume 1 µL
Oven Temperature Profile:
Initial Temp 100°C, hold for 1.0 min
100°C to 150°C at 3°C/min
150°C to 220°C at 1°C/min
220°C to 280°C at 5°C/min, hold for 3 min
NOTE: For PBDE analysis, the final hold is extended to 13 mins.

26.5.2. Standards, see section 9.3. and 9.5.

26.5.3. Instrument Performance: Response factors are generated from a run of the calibration standard. This standard will be run every 12 hours as a performance standard and evaluated for resolution, reproducibility, and sensitivity. The calculated concentrations of PCB congeners #26 and #199 (small peaks) shall not differ from their known concentrations by more than 40%, and those of congeners #101, #185, #6, #70/76, and #180 (average and large peaks), shall not differ by more than 20%. If these limits are exceeded, response factors will be re-generated, or the necessary instrument maintenance will be performed.

26.5.4. Samples: All samples are screened by packed column GC-EC to insure adequate clean-up, and are diluted or concentrated to an appropriate volume for injection onto the capillary column. LODs are based on 40 g dry weight and an extract volume of 10 mL, or equivalent. Internal standards are added to the cleaned-up sample extract just prior to capillary column gas chromatography. Twenty-five µL of a standard containing Congener #30 at 0.568 mg/L and congener #204 at 0.624 mg/L are added to an exactly known fraction of the extract (usually 1.0 mL). This results in a mass of 14.2 ng of congener #30 and 15.6 ng of congener #204 added. The sample size (weight) represented by the portion of extract to which internal standards are added must be exactly known.

26.5.5. Calculations

26.5.5.1. Calculations are done by the HPChemstation integrator, using the formula for internal standard quantitation:

$$\text{Conc.} = \frac{\text{Response (y)}}{\text{Response (IS)}} \times \frac{\text{RF (y)}}{\text{RF (IS)}} \times \text{Amt (IS)} \times \text{Mult.}$$

where: y = congener
IS = internal standard
RF = response factor = mass / response
Amt (IS) = mass of internal standard added to the sample
Mult. = multiplier = 1 / sample size

26.5.5.2. Response factors are generated from a current run of the calibration standard. "Amt (IS)" and multiplier are entered in the sample table. The concentration units are thus determined by the units used for "Amt (IS)" and sample size.

26.5.6. Confirmation of correct PCB and PBDE identification is done on 5% of the samples using retention time agreement on a 60 m DB-1 column, using the same standards and GC conditions as given in Section 10.5.1.

26.6. Gas Chromatography for "Toxic" Congeners using a Siemens Multidimensional Gas Chromatograph

26.6.1. Theory: Certain PCB congeners which are not adequately separated on a single capillary column can be separated using multi-dimensional or "heart-

cutting" gas chromatography. On the Siemens system, components in a mixture are separated on two capillary columns in series, each one in an independently-controlled GC oven, and each with an electron capture detector. A "live T-piece" switching device utilizing pressure differentials quantitatively diverts narrow pre-selected "cuts", containing two to three coeluting components from the first column to the second column. These components are separated and then detected on the second detector. (See references 15.3 and 15.4.)

26.6.2. GC Conditions: Siemens SiCHROMAT-8 Gas Chromatograph

	Channel A	Channel B
Columns:	30M x .32mm DB-5 0.25 µm film	30M x .32mm DB-210 0.25 µm film
Detectors:	Electron Capture Ni63 @ 330°C	Electron Capture Ni63 @ 330°C
Carrier Gas:	Hydrogen	Hydrogen
Oven Programs:		
Init. Temp	90°C ; hold 1 min.	130°C hold 0 min
Prog. Rate 1	5°C /min	1°C /min
Final Temp 1	150°C ; 0 hold	215°C ; hold 0 min.
Prog. Rate 2	2°C /min.	-25°C /min.
Final Temp 2	250°C ; hold 0 min.	130°C ; hold 0 min.
Prog. Rate 3	15°C /min	
Final Temp 3	310°C	
Prog. Rate 4	-20°C /min.	
Final Temp 4	90°C	
Injector Parameters:	Channel A Only	
Injector Temp:	300°C	
Inj. Speed:	60 cm/sec.	
Inj. Volume:	2 µL (40%)	
	No Bubble	
	No Solvent Plug	
Purge Valve ON at 1.0 min.		

26.6.3. Cuts to Column B: The following times for cuts were determined by injecting standards, determining retention times on Channel A and progressively adding cuts starting with the earliest peak. These times may need to be adjusted periodically as retention times on Channel A shift slightly with continuous use. See the instrument operating procedure (ESS IOP 0150) for guidance.

Time Table for Cuts from Column A to Column B

Congener	Cut ON min.	Cut OFF min.	Congener	Cut ON min.	Cut OFF min.
ISTD #30	21.57	22.07	167	48.64	49.20
81	38.48	39.08	156	50.55	51.00
77	39.39	40.04	157/204	51.10	51.85
123/118	41.48	42.27	180	52.10	52.70
114	42.58	43.18	169	53.90	54.54
105	43.92	44.52	189	57.10	57.70
126	46.80	47.40			

NOTE: GC Method 1 includes all the cuts. GC Method 2 contains the cuts listed in section 9.4. Both methods use the GC conditions given in section 10.6.2

26.6.4. Standards: The calibration standard is a mix containing these 13 congeners at concentrations of 10 ng/mL (GC Method 1), or 5.0 ng/mL (GC Method 2), plus #30 as Internal standard at 5.68 ng/ml and #204 as Retention time reference peak.

26.6.5. Instrument Performance: Response factors are generated from a run of the calibration standard before a sequence of sample injections. A re-calibration run is made after 24 to 48 hours of runs in a sequence and whenever the set of cuts (GC Method) is changed or if the GC response changes significantly. Response factors should generally not change by more than 15% during a sequence of runs. A calibration check at a concentration from 1.0 to 15 ng/mL is run after every 6 to 8 samples.

26.6.6. Samples: Ten (10) µL of Internal Standards containing PCB #30 at 0.568 mg/L and PCB #204 at 0.624 mg/L are added to an exactly known fraction of the sample extract, usually 1.0 mL. This results in a mass of 5.68 ng of PCB #30 and 6.24 ng of #204 added. Detection limits for PCBs #81, #77, #114, #126, #167, #157, #169 and #189 are based on 40 g dry weight and an extract volume of 5.0 mL or equivalent. To minimize interferences on the B column, GC Method 2 with only these cuts should be used. The remaining congeners can be analyzed at a volume of 10 mL for 40 g of sample or equivalent, GC Method 1 with all cuts, and with further dilutions as required to keep sample responses within the linear range.

26.6.7. Calculations: Calculations are done by the Spectra Physics integrator using the internal standard quantitation method. Sample weight, scale factor (extract volume) and mass of Internal Standard are entered into the Spectra Physics sample table.

27. Calculations: See the above Section 10 for calculations

28. Data Management: Data is collected using either an HP3396 integrator, SP4270 integrator, or a PC-based HPChemstation. PCB Congener data is also collected in a computer file, reviewed by the analyst, and electronically transferred to the Laboratory's LIMS system. It is then reviewed by peers or the section supervisor before being released. Pesticide, Aroclor, PBDE and PCB toxic congener data is transcribed by the analyst onto the sample worksheet. It is then reviewed (by peers or section supervisor) and manually entered into the Laboratory's LIMS system.

29. Definitions: General definitions of other terms that may be used in this method are found in Section 19 of the SLH Quality Assurance Manual.

30. Method Performance: Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure "ESS ORG QA0012 LOD and LOQ Determinations". Data generated within the last two years will be located in the filing cabinet in the Department supervisor's cubicle. Any data older than two years is stored in the Department filing cabinet in the basement.

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31.11. "Quality Assurance Procedures and Policies", The ESS QA Manual.

31.12. "Constitution, Bylaws, and Standards", National Environmental Laboratory Accreditation Conference, (July 1999)

32. Tables, figures, diagrams, charts, checklists, appendices: See following Pages

Table I

PCB Congener Calibration Concentrations - DB-5 Column

#	NAME	AMOUNT, ng/mL	#	NAME	AMOUNT, ng/mL
1	#1	3.6000E+01	42	#83	4.5000E-01
2	#3	2.1000E+01	43	#97	1.6800E+00
3	#4/10	1.0200E+01	44	#87	3.0000E+00
4	#7/9	3.6000E+00	45	#85	2.1000E+00
5	#6	5.7000E+00	46	#136	2.2500E+00
6	#8/5	4.2000E+01	47	#77/110	6.4000E+00
7	#14	3.1600E+01	48	#82	1.3200E+00
8	#19	8.4000E-01	49	#151	5.1000E+00
9	ISTD 1#30	1.4200E+01	50	#135/144	2.6700E+00
10	#18	1.1100E+01	51	#123/149	8.4000E+00
11	#15/17	1.1100E+01	52	#118	3.6000E+00
12	#24/27	7.8000E-01	53	#146	1.1700E+00
13	#16/32	1.1700E+01	54	#132/153/105	1.2900E+01
14	#26	2.1600E+00	55	#141	5.1000E+00
15	#25	9.6000E-01	56	#137/176	7.8000E-01
16	#28/31	2.8200E+01	57	#163/138	8.1000E+00
17	#33	9.9000E+00	58	#158	7.5000E-01
18	#53	1.9200E+00	59	#178	3.3000E+00
19	#51	5.4000E-01	60	#166	8.4000E+00
20	#22	8.7000E+00	61	#187/182	1.0800E+01
21	#45	2.6700E+00	62	#183	5.1000E+00
22	#46	1.2000E+00	63	#128	4.3000E+00
23	#52	1.3500E+01	64	#167	1.9000E+00
24	#49	6.9000E+00	65	#185	1.4000E+00
25	#47/48	6.0000E+00	66	#174	9.6000E+00
26	#65	7.8000E+00	67	#177	5.1000E+00
27	#44	1.2900E+01	68	#202/171	2.3700E+00
28	#37/42	7.8000E+00	69	#157/200	1.1700E+00
29	#41/71/64	1.2300E+01	70	ISTD 2#204	1.5600E+01
30	#40	2.8000E+00	71	#172	1.6800E+00
31	#63	6.3000E-01	72	#180	1.8300E+01
32	#74	5.7000E+00	73	#193	1.2600E+00
33	#70/76	1.0200E+01	74	#199	1.3000E+00
34	#66	1.5600E+01	75	#170/190	5.1000E+00
35	#95	6.0000E+00	76	#198	3.6000E-01
36	#91	1.5300E+00	77	#201	1.3000E+01
37	#56/60	1.0500E+01	78	#203/196	1.3000E+01

38	#92/84	5.4000E+00	79	#208/195	2.4000E+00
39	#89	3.0000E-01	80	#207	2.8000E-01
40	#101	5.4000E+00	81	#194	5.4000E+00
41	#99	2.2200E+00	82	#206	2.0000E+00

Table II

PCB Stock Solution Concentrations -- 183 µg/mL

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CALCULATED AVERAGE

Peak Name	Calc'd Congener Conc'ns µg/mL	Peak Name	Calc'd Congener Conc'ns µg/mL	Peak Name	Calc'd Conc'ns µg/mL
PCB-000	4.1	PCB-064	1.8	PCB-163+138(SUM)	2.7
PCB-001	12	PCB-066	5.2	PCB-167	0.049
PCB-003	7.0	PCB-070+076(SUM)	3.4	PCB-170+190(SUM)	1.7
PCB-004+010(SUM)	3.4	PCB-074	1.9	PCB-172	0.56
PCB-006	1.9	PCB-077	0.23	PCB-173	0.038
PCB-007+009(SUM)	1.2	PCB-081	0.16	PCB-174	3.2
PCB-008+005(SUM)	14	PCB-082	0.44	PCB-175	0.20
PCB-012	0.17	PCB-083	0.15	PCB-177	1.7
PCB-013	0.097	PCB-085	0.70	PCB-178	1.1
PCB-015+017(SUM)	3.7	PCB-087	1.0	PCB-180	6.1
PCB-016	2.0	PCB-089	0.10	PCB-183	1.7
PCB-018	3.7	PCB-091	0.51	PCB-185	0.47
PCB-019	0.28	PCB-092+084(SUM)	1.8	PCB-187+182(AVE)	3.6
PCB-021	0.032	PCB-095	2.0	PCB-189	0.040
PCB-022	2.9	PCB-097	0.56	PCB-191	0.12
PCB-024+027(SUM)	0.26	PCB-099	0.74	PCB-193	0.42
PCB-025	0.32	PCB-100	0.11	PCB-194	1.8
PCB-026	0.72	PCB-101	1.8	PCB-197	0.11
PCB-029	0.053	PCB-107	0.13	PCB-198	0.12
PCB-031+028(SUM)	9.4	PCB-110	1.9	PCB-199	0.43
PCB-032	1.9	PCB-114+131(SUM)	0.14	PCB-201	4.2
PCB-033	3.3	PCB-118	1.2	PCB-202+171(AVE)	0.79
PCB-037	1.2	PCB-119	0.028	PCB-203+196(SUM)	4.3
PCB-040	0.94	PCB-123+149(SUM)	2.8	PCB-205	0.11
PCB-041+071(AVE)	2.3	PCB-128	0.10	PCB-206	0.68
PCB-042	1.4	PCB-129	0.013	PCB-207	0.093
PCB-043	0.27	PCB-130	0.075	PCB-208+195(SUM)	0.80
PCB-044	4.3	PCB-132+153+105(SUM)	4.3	PCB-209	0.012
PCB-045	0.89	PCB-134R	0.072		

PCB-046	0.40	PCB-135+144(SUM)	0.89
PCB-047	1.0	PCB-136	0.75
PCB-048	1.0	PCB-137+176(AVE)	0.26
PCB-049	2.3	PCB-141	1.7
PCB-051	0.18	PCB-146	0.39
PCB-052	4.5	PCB-151	1.7
PCB-053	0.64	PCB-156	0.066
PCB-056+060(AVE)	3.5	PCB-157+200(AVE)	0.39
PCB-063	0.21	PCB-158	0.25

33. Signatory Page:

33.1. Written by: Carol Buelow Date: 10/6/03
Title: Chemist-Advanced
Unit: ESS Organic Chemistry

33.2. Reviewed by: Carol Buelow Date: 7/6/04
Title: Chemist-Advanced
Unit: ESS Organic Chemistry

33.3. Approved by: Steve Geis Date: 10/7/04
Title: ESS Organic Supervisor
Unit: ESS Organic Chemistry

APPENDIX F

TOXICITY DATA AVAILABLE TO DATE

Summary of Aquatic Toxicity Data Available to Date

Common Name	Species Name	Chemical	Duration/Endpoint	Toxicity Value	Reference
Algae	*Raphidocelis subcapitata*	BDE-99	inhibition of growth	>100 µM	Evandri et al. 2003
Water flea	*Daphnia magna*	BDE-99	48-h EC50	0.044 µM	Evandri et al. 2003
Japanese rice fish	*Oryzias latipes*	Commerc. Penta mix	48-h LC50	= 500,000 µg/L	in Hardy 2002
Rainbow trout	*Oncorhynchus mykiss*	Commerc. Penta mix	96-h LC50	= water solubility	in Hardy 2002[a]
Rainbow trout	*Oncorhynchus mykiss*	Commerc. Penta mix	ELS NOEC	~ water solubility	in Hardy 2002[b]
Rainbow trout	*Oncorhynchus mykiss*	Commerc. Penta mix	Juvenile NOEC	8.9 µg/L	in Hardy 2002[b]
Rainbow trout	*Oncorhynchus mykiss*	Commerc. Penta mix	Juvenile LOEC	16 µg/L	in Hardy 2002[b]
Algae	*Selenastrum capricornutum*	Commerc. Penta mix	96-h EC50	= water solubility	in Hardy 2002[c]
Water flea	*Daphnia magna*	Commerc. Penta mix	48-h EC50	14 µg/L	in Hardy 2002[d]
Water flea	*Daphnia magna*	Commerc. Penta mix	48-h NOEC	4.9 µg/L	in Hardy 2002[d]
Water flea	*Daphnia magna*	Commerc. Penta mix	21-d LOEC	9.8 µg/L	in Hardy 2002[d]
Water flea	*Daphnia magna*	Commerc. Penta mix	21-d NOEC	5.2 µg/L	in Hardy 2002[d]
Brackish copepod	*Nitocra spinipes*	BDE-47	96-h LC50	4,400 µg/L	Breitholtz et al. 2001
Marine copepod	*Acartia tonsa*	BDE-47	48-h LC50	2,370 µg/L	Breitholtz et al. 2001
Marine copepod	*Acartia tonsa*	BDE-47	5-d EC50	13 µg/L	Breitholtz et al. 2001
Marine copepod	*Acartia tonsa*	BDE-47	ACR (LC50/EC50)	182	Breitholtz et al. 2001

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APPENDIX G

PBDE REFERENCES

PBDE Reference List
(includes abstracts, proposals and unpublished reports)

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